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Title: EXOGENOUS AND ENDOGENOUS FACTORS RELATED TO SEED GERMINATION AND VIGOR IN CERTAIN VARIETIES OF SUGARBEET BETA VULGARIS L.

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Studies were conducted on the causes of low germination of monogerm sugarbeet seed grown in western Oregon. Emphasis was placed on the identification and measurement of endogenous inhibitors as related to the germination and vigor. Both qualitative and quantitative analytical methods were applied to examine the organic substances in the aqueous seedball extracts as influenced by environmental factors during maturation. Effects of exogenous factors such as harvest time after anthesis, liming of the soil and post harvest drying temperature on the subsequent germination were examined.

Attempts were made to improve germination potential of a given seed lot by pre-germination treatments. Effects of soaking, leaching and drying upon germination were compared and examined. Leaching studies were further expanded to measure the change of

inhibitor content at various leaching intervals and to relate concentrations to germination and vigor. Varying concentrations of gibberellic acid (potassium salt) were applied to germinating seed to examine effects on seed dormancy.

Five phenolic compounds and oxalic acid as the soluble and insoluble salt were shown to be present in the sugarbeet fruit. Among the five known phenolic substances, ferulic acid was most inhibitory to germination. Oxalic acid at various levels of concentration did not influence germination although oxalic acid did inhibit the seedling growth at relatively low concentrations.

Among four sugarbeet seed samples tested, a low germinating lot (variety 4426) was shown to contain the least amount of oxalic acid, however, ferulic acid content of this lot was considerably higher than the others. It was concluded that oxalic acid was not involved in dormancy of the seed samples tested.

Among the several pre-germination treatments tested to improve germination potential of the given varieties, complete drying after leaching of seed samples showed the best response. Germination potential of the monogerm varieties used in these studies greatly increased by drying them completely after leaching. Vigor as measured by length of seedlings was markedly improved. Simple leaching did not improve germination. Optimum leaching time for best germination of the varieties tested was from 12 to 20 hours.

Exogenous and Endogenous Factors Related to Seed Germination and Vigor in Certain Varieties of Sugarbeet *Beta vulgaris* L.

by

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EXOGENOUS AND ENDOGENOUS FACTORS RELATED TO SEED GERMINATION AND VIGOR IN CERTAIN VARIETIES OF SUGARBEET (Beta vulgaris L.)

I. INTRODUCTION

The sugarbeet (Beta vulgaris L.) is a biennial plant and normally requires two seasons for the production of seed. The reproductive phase of growth, with the production of seedstalk and flowering branches, can be induced to the vegetative phase by cool temperatures. Most commercial varieties of sugarbeets require 90 to 110 days of exposure to low inductive temperature for reproductive development. In areas where winters are mild enough to permit survival of the plants an excellent yield of seed can be obtained by growing the sugarbeet plants as winter-annual (fall-sown plants that bolt, bloom and fruit the following spring). However, the period of cool temperatures must be long enough to supply the photothermal induction required for reproductive growth the following spring.

The Willamette Valley of Western Oregon has relatively long mild winters and is well suited for the production of most commercial varieties grown in the United States. The sugarbeet seed industry in this area, therefore, has been expanded from 1,470 acres harvested in 1941 to 2,336 acres harvested in 1967, with an annual record production of nearly five million pounds of seed. Since the American sugarbeet industry has become wholly dependent on domestic seed

production in recent years, the sugarbeet seed industry in Oregon has a promising future.

In most years, sugarbeet seeds grown in the Willamette Valley have a germination rate of less than 80 percent with some variation depending on the variety and the year of planting. Since the introduction of monogerm varieties for commercial production a decade ago, the problem of low germination has become more critical simply because the number of seed per seedball decreased from two or three to one.

Germination tests have shown that a significant number of sound plump seeds failed to germinate in intact seed lots and occasionally these nongerminated seed constitute a sizeable percentage of the seeds. Many of these sound seeds germinate after being leached with water. This observation suggests that some inhibitory substance or substances has been removed by leaching.

Germination inhibitors extracted from sugarbeet seeds have been shown to be effective on other plant seeds. However, no laboratory study has yet been reported on the hypothesis that these inhibitors prevent germination of the sugarbeet seed itself.

The objectives of this research were to: 1) examine the chemical inhibitors present in sugarbeet fruits grown in Oregon, 2) relate the level of various inhibitors to germination and vigor, 3) determine environmental factors affecting inhibitor level and

germination, and 4) evaluate means of removing seed dormancy. Samples of low and high germinating seed were compared.

Studies were also conducted on effects of exogenous factors such as maturity, temperature, and synthetic growth substances, on the germination potential of monogerm sugarbeet seed. Finally efforts were directed to the effects of leaching on germination as a way of eliminating chemical inhibitors.

II. LITERATURE REVIEW

In addition to the growth promoting substances first isolated by Went in 1921 from the tips of Avena coleoptiles (47), there also exists in plants, substances that inhibit growth and germination (10, 26, 29). According to Oppenheimer it was Molisch who first suggested that the juices of fruits retard the germination of seeds because of the presence of inhibiting substances (51). Subsequently, it has been established that many fruits and seeds do, in fact, contain substances which, when extracted and applied to suitable test seeds, markedly inhibit growth and germination. These substances have been reviewed by Evenari (13), Toole et al. (45), and Wareing (51, 52). The inhibitors may be present in various parts of the fruits, including succulent and non-succulent pericarp tissue, endosperm, testa, and embryo.

The most common inhibitors are aromatic organic compounds; although there are also such diverse inhibitors as ascorbic acid (44), some fatty acids (5), and metallic ions (9). The simple phenyl compounds are most widely represented; these include the phenols, the benzoic acids, the longer-chained cinnamic acid series, and the lactones of these, the coumarin compounds (see Figure 1). Large molecules have also been found to be inhibitory to growth. These include the flavonoids and their derivatives.

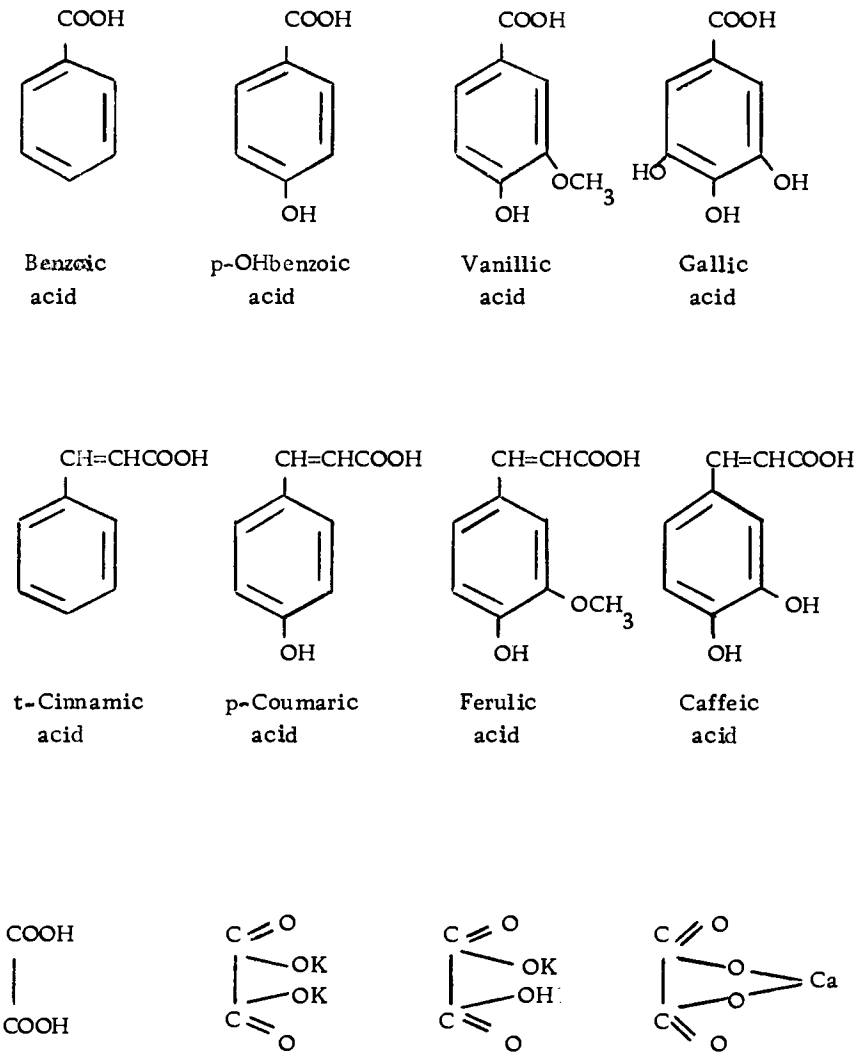

Figure 1. Structural formula of inhibitors known to be present in sugarbeet fruits. Derivatives of benzoic acid including p-hydroxy benzoic, vanillic and gallic acids are very common, especially in ripening fruits. The cinnamic acid derivatives include the cofactors for indoleacetic oxidase, p-coumaric acid and ferulic acid. Oxalic acid and its derivatives, potassium and possibly sodium oxalate have been reported as germination inhibitor in sugarbeet seed.

Germination Inhibitors in Sugarbeet Fruits

At least a dozen substances which may potentially be inhibitory to germination have been isolated from the fruits of sugarbeets (11, 12, 29, 32, 39, 42, 47). Most of these compounds are organic in nature and some of the compounds' chemical structures have not been identified. There is only limited data on the quantity of these inhibitors in sugarbeet fruits at the present time.

Duym et al. (38) have reported that the osmotic pressure exerted by inorganic substances in the seedball of the sugarbeet is responsible for the inhibitory effect on germination. Specific conductance values of different seedball extracts were later measured by Snyder (38). These aqueous extracts were variable in the amount of electrolytes and these differences were reflected by the wheat growth test (39). Froeschel (17) observed that aqueous extracts of sugarbeet fruits greatly inhibited germination of Lepidium spp. and other seeds. Later aqueous extracts made by Massart was shown to emit volatile inhibitory substances. His data indicate that specific organic substances were the cause of inhibition (26).

Stout and Tolman (41) reported that sugarbeet fruits contain a toxic substance which varies in amount from one variety to another, and within a variety from year to year depending on soil and climatic conditions at maturity. They later reported that this substance was

Ammonia (42). However, De Kock and his co-workers (11) did not feel that excess ammonia was the cause of poor germination. They isolated a yellow oily substance from the water extract of beet seeds which acted as a powerful germination inhibitor when tested on Lepidium and other seeds.

According to Makino and Miyamoto (25, 29), water soluble oxalate, which may exceed two percent in the fruits of some sugarbeet varieties, is the principle inhibitor in sugarbeet seeds.

Massart and his co-workers (26) analyzed the aqueous extracts for phenolic acids and reported the presence of p-hydroxybenzoic, p-coumaric, ferulic and vanillic acid in the sugarbeet seeds.

Koves and co-worker (21) also demonstrated the presence of the above compounds with the exception of vanillic acid. Besides the identified compounds, the presence of other inhibitors was noted. Presumably these were chemicals related to the substances mentioned above. After making a comparison of the inhibitors in dry fruits with those demonstrated from fresh fruits, they concluded that phenolic acids were common inhibiting substances in higher plants.

Van Sumere (47) analyzed sugarbeet seed extracts and confirmed the presence of the same organic compounds identified by Massart and co-workers. He also showed that pure solutions of ferulic acid gave strong inhibition of cress seed at concentrations as low as 10^{-3}M and 10^{-4}M. He concluded that ferulic acid was the most

effective germination inhibitor identified in the sugarbeet extract.

Snyder and co-workers (39) later reported that among the organic substances present in the seedball extracts, only oxalate had been causally related to speed of germination. A study of the distribution of oxalate in the sugarbeet fruit revealed that oxalate was concentrated in the corky material of the fruit. They also stated that the inhibition was caused largely by the presence of organic substances. Recently, Snyder and his co-workers (37) reported that gallic acid had been recovered from a water extract of sugarbeet fruits along with ferulic, caffeic, vanillic and p-hydroxybenzoic acid. They also indicated that gallic acid at 10^{-3}M concentration showed the greatest inhibitory action on lettuce seed germination.

The Interaction of Inhibitors with Other Factors

Temperature

When lettuce seeds are germinated in a series of different constant temperatures in darkness their requirement for light as a condition for full germination is more or less abolished at lower temperatures (14). When germinated in coumarin, it becomes clear that lower temperatures counteract the inhibiting influence while high temperatures enhance the inhibition.

The fact that high temperatures cause growth inhibition and

dormancy, has occasionally been attributed to elevated inhibitor concentration (49). Low temperature can reduce the inhibitor content and is a component part of the improvement of seed germination by stratification.

Several researchers have studied dormancy in Fraxinus in relation to inhibitors (2, 15). Ferenczy (15) using crude extracts of various parts of the fruits of F. excelsior concluded that most of the inhibitory material is present in a mucilaginous layer surrounding the seeds. He found a decrease in this inhibitory material during the moist storage at both 20°C and 5°C. On the other hand, Villiers and Wareing (50) found that the endosperm and embryo of F. excelsior had a relatively high inhibitor content. It was found that there was little change in the inhibitor content during chilling periods. Nevertheless, the chilling process very effectively breaks seed dormancy. On the other hand, it was found that leaching is necessary to induce the germination of unchilled embryos of F. excelsior. If unchilled embryos were maintained in a moist atmosphere under conditions which do not permit leaching; they remained dormant for long periods. If such embryos are first leached in water, unchilled embryos give rise to dwarf seedlings, whereas chilled embryos give rise to normal seedlings.

Although chilling does not reduce the inhibitor content of the seeds of F. excelsior, it was found that chilled embryos contain a

germination promotor which is absent in unchilled embryos. Thus, the control of dormancy and germination in this seed appears to involve an interaction between an inhibitor and a promotor (50).

Light

There are many effects of light on growth inhibitors. The level of inhibitors is generally lower under long days than short days (20). The fact nevertheless remains that, in many species, inhibitors are present under long days. The reason why short photoperiods result in more inhibitors than the long photoperiods is unknown. Perhaps more precise evidence supporting the role of light is shown in the experiment by Masuda (27) who compared the inhibitor contents of etiolated seedlings. He observed a marked increase in germination after light treatment.

Light may be able to overcome some inhibitor actions. This is implied from the fact that it can overcome dormancy. Nutile (32) made the original observation that the inhibitory effect of coumarin on lettuce seeds can be overcome by exposure to light. A detailed study of the interaction between the effects of light and coumarin has been made by Evenari (14).

Interactions between light effects and endogenous inhibitors have been studied in birch seed (Betula verrucosa). Unchilled birch seeds require light; germination is nil in complete darkness. The

light responses are greatly modified by temperature. At 20°C and above, a single period of eight illuminated hours is sufficient to allow high germination; but at 15°C the germination is markedly photoperiodic and there is little germination with repeated eight hour-photoperiods. Exposure to long-day cycles for eight days results in a high germination (46).

Promotors

Considering the possible role of inhibitors in the breaking of dormancy by chilling, it has been shown for several woody species that the level of inhibitor in the buds decreases progressively during the winter and reaches a minimum about the time of bud-break. With this evidence it might be concluded that the effect of chilling is brought about by removal of the endogenous inhibitors. However, it has been found that chilling of several types of seed seems to result in very little change in the levels of inhibitor (50). It has been shown that there is an increase in the level of growth-promoting substances during chilling of F. excelsior. Villiers (50) was able to extract a fractionate from chilled embryos of F. excelsior which was capable of stimulating the germination of dormant embryos of the same species. It has been shown that when seedlings of woody plants are kept under different photo-periodic conditions, there are less inhibitors and greater amounts of growth promoting substances under

long days (31). Not all of the promoting substances change equally in response to photoperiod; some fractions change more than others. Similarly, chilling of the seed of Fagus sylvatica does not affect the overall gibberellin activity, but results in an increase in one fraction and a decrease in another (16).

These observations suggest that dormancy may be brought about by inhibitors and that the removal of dormancy by chilling may depend upon a build-up of promoting substances which overcome the effect of the inhibitors.

Many natural inhibitors in plant extracts have a synergistic effect on growth; promoting auxin-induced growth at low concentrations and inhibiting at higher concentrations (22). This complicated effect was noted by Thimann and Bonner (44) for the lactones, such as coumarin. It has been suggested that synergistic germination effects may be due to competition of auxin and courmarin for active sites, causing a greater efficiency of the auxin at very low inhibitor concentrations.

While the information on interaction of auxins has evolved into a complicated array dealing with competition and synergism the interactions of inhibitors with other growth regulators have hardly been examined.

Oxygen

Several properties of the seedcoat have been described (52) which could greatly retard the diffusion of oxygen to the embryo: a) the occurrence of an aleurone-like layer which may be the site of high respiratory activity, b) the consumption of oxygen during oxidation of phenols, and c) the additional resistance to diffusion of oxygen which arises as a result of the swelling of the mucilagious contents of the epidermis on wetting (51).

In certain instances there is good evidence that the primary effect of the seedcoat is due to interference with gaseous exchange. It can be shown that simply pricking the seedcoat is sufficient to bring about germination. This was observed not only in the case of Xanthium, where dormancy had long been thought to be due to interference with oxygen uptake by the testa, but also in light-requiring seeds such as birch and lettuce (6), and seeds with a chilling requirement (51).

On the other hand, it has been reported that the dormancy effects cannot be interpreted entirely in terms of interference with gaseous exchange. Wareing and co-workers (52) have been able to induce growth of dormant embryo in Xanthium and Avena fatua either by leaching out the inhibitor or by placing them in an atmosphere of high oxygen concentration. They reported that the requirement for

high oxygen concentration could be due not merely to physical barriers, but also to the presence of inhibitors (51).

Maturation of Seeds

Inhibitor contents of leaves, stems, fruits and roots increase with age (48). A clear case of an increase in growth inhibitors related to photoperiodism was reported by Kawase (20) on photoperiodic effects of Betula. The suppression of growth by photoperiods has been attributed to the formation of inhibitors in the leaves with the presumed translocation of the inhibitor to the growing points (35).

A study of the inhibitor content of the embryo of Xanthium during seed development (52) showed that during the early stages there is no inhibitor present in the embryo; however the inhibitors gradually accumulate during the later stages of development and reach a maximum when the embryo ceased to grow.

Biological Functions of Germination Inhibitors

Although the knowledge about the mode of action by germination inhibitors is quite limited, the biological and ecological importance of these compounds are quite clear. It has been discussed by Evenari (13, 14), who pointed out that the occurrence of germination inhibitors in the seed or fruit is the basic component of the endogenous control of plant growth and development.

These inhibitors are very important in controlling germination, which is one of the most important factors of survival of the species. The presence of germination inhibitors in the seed or fruit tends to result in sporadic germination over extended periods of time. Where seeds or fruits contain inhibitors, the latter are removed by physical, chemical and biological action at different rates; this results in irregular germination over an extended period. In this way, the risk of total extinction of the species is greatly diminished.

Another function of germination inhibitors is apparently to confine germination to suitable habitats. In some desert species germination occurs only after a certain quantity of rain has fallen (53), and it appears that this requirement for a certain minimum rainfall is determined by the amount of rain required to leach out the inhibitors.

It appears that the leaching of water-soluble inhibitors from the seeds of certain species prevents the establishment of other species in their immediate surroundings, thus keeping their habitat free of possible competitors (8, 14, 22).

III. METHODS, MATERIALS AND RESULTS

General Methods and Materials

Seed Samples

Samples of sugarbeet varieties grown commercially in Oregon, were used in these experiments. AH-1, USH-9B and HH-5 were obtained from West Coast Beet Seed Company, Salem, Oregon. The remaining samples, including variety 4426 which has low germination potential, were made available through the courtesy of Dennis M. Tekrony of the Seed Laboratory, Farm Crops Department, Oregon State University. Variety 569H3 was grown at the East Farm of the Farm Crops Department and used in maturity-germination studies.

Chemicals

Reagent grade phenolic compounds used were all known to be present in sugarbeet fruit. They are: Vanillic acid (4-hydroxy-3-methoxy benzoic acid), ferulic acid (4-hydroxy-3-methoxy cinnamic acid), t-cinnamic acid, p-hydroxybenzoic acid, and gallic acid.

In addition the following technical grade compounds were used for the identification of unknown substances in the sugarbeet seeds: Flavonoids, oxalic acid and its potassium salt.

Many of the above compounds (except ferulic and vanillic acid)

and other chemicals including inorganic acids and organic solvents were obtained from the chemistry store, Oregon State University.

Germination Techniques

Four 100 seed samples for each treatment were germinated on two sheets of Whatman No. 1 filter paper in clean petri-dishes using either distilled water or various solutions of chemicals or aqueous extracts of sugarbeet fruits at a temperature of about 20 (±2)°C. The petri-dishes were kept moist and maintained in the dark until completion of each experiment; and the germination media added at regular intervals. In many instances, the seeds were placed on the surfaces of filter paper without pre-soaking treatment in order to avoid the loss of inhibiting substances.

Seed germination was recorded daily for seven to ten days and the germinated seeds were measured at the final count for average length of seedling per treatment. After completion of each germination trial, all the ungerminated seeds in each treatment were cut in half with a dissecting knife and were then separated into three classes i.e., normal, abnormal and empty. The normals included the firm-ungerminated seeds and seeds which had a visible embryo with a large amount of chalky perisperm. The abnormals included fruits having decayed seeds or those having partly developed shrunken seeds. The empties included fruits having no seed or those having

only testa.

Details of germination will be described later for each experiment.

Vigor Measurement

Seedling length, both radicle and shoot, was measured to the nearest millimeter seven to ten days after planting. Usually 40 seedlings were randomly selected from each treatment and the mean seedling length was used as the criteria of seedling vigor.

Experimental Procedures and Results

Studies related to germination inhibitors were grouped in nine categories.

The least significant differences were determined from an analysis of variance. Mean squares comparing varieties, treatments and collections are shown in the Appendix.

Experiment 1. Water Soaking as a Method of Relieving Dormancy in Sugarbeet Seed

This experiment was designed to evaluate various methods of soaking on subsequent seed germination. Although the presence of germination inhibitors in the sugarbeet fruit has long been recognized, methods of improving germination potential of a given seed

lot are not completely understood.

Methods and Materials

A sample of USH-9B was used for this experiment. Seeds were divided into four different size groups i.e., extra small, small, medium and large. Extra small seeds included those passed through No. 8 screen, small included those retained by No. 8 screen but passed through No. 9 screen, medium seeds included those retained by No. 9 screen but passed through No. 12, and those retained by No. 12 screen were classified as large seed. Thirty seeds were taken from each group for treatment 1 and 2 making a total of 120 seeds per treatment. For treatment 3, 50 seeds were selected from the large, medium and small size groups with a total number of 150 seeds. Sample seeds in each treatment were separately bagged in a 3" x 3" cheese cloth and were treated as follows:

Treatment 1. A bagged sample was placed in 250 ml beaker and placed under the flowing tap-water (flow rate of approximately 1 liter per minute).

Treatment 2. Another bagged sample was placed in 150 ml of distilled water in a 250 ml beaker.

Treatment 3. The last bag was placed in 150 ml of distilled water in a 250 ml beaker. Air was bubbled from the bottom of the beaker in an attempt to create high oxygen concentration. In all

cases, the sample bags were kept in a submerged condition by placing a small glass plate on top of the bag.

Results

The data for this experiment are summarized in Table 1. The results show that germination is increased by leaching out certain substances from the sugarbeet seeds. With limited water supply, no germination was observed in a seven day period; however, when air (or oxygen) was supplied to the seeds in a limited amount of water, a majority of the seeds germinated.

The ungerminated seeds from each treatment were later placed in the petri-dishes for germination. Seeds from treatment 1 and 2 showed less than a 3% increase in germination while treatment 2 gave an average of 43 percent.

Table 1. Germination of monogerm sugarbeet seed under submerged conditions and in subsequent petri-dish tests.

Treatment	% Germination		
	Submerged	Petri-dish tests	Total
1. Flow-water[1]	82.5	2.5	85.0
2. Still-water[1]	0	43.0	43.0
3. Still-water[2] with air	81.0	1.0	82.0

[1]Average of four samples, 30 seeds per sample.

[2]Average of three samples, 50 seeds per sample.

Inhibitors may be active in the seeds in treatment 2, and the inhibitory effect may be removed from the seeds by either leaching or oxidation.

Experiment 2. Comparative Studies of Water and Organic Solvent Extracts of Sugarbeet Fruit to Determine Inhibitory Effects on Germination

This experiment was designed primarily to compare various solvent extracts of sugarbeet fruit as germination inhibitors.

Methods and Materials

Commercial monogerm variety, AH-1, was used for extraction. About 100 grams of sample seeds were ground in a motar and an equal amount of finely ground sample was extracted with various organic solvents. The solvents used in the experiments were reagent grade methanol, acetone, ether and 95% ethanol. Five successive extractions at room temperature were made with the respective solvent. The extracts were then concentrated by distillation in a vacuum at 35^{o}C. The concentrates were dissolved in an equal amount of distilled water for germination tests.

Flavonoid compounds were extracted from sugarbeet fruits using the techniques described by Bate-Smith (4). The isolated flavonoid mixture was completely vacuum evaporated at 35^{o}C and the residue was extracted with ether. Both the ether soluble portion and

the insoluble portion of the flavonoid compounds were again evaporated to complete dryness. The dried portions of the flavonoids were used for germination by dissolving them in equal amounts of distilled water.

The aqueous extracts were prepared by soaking ground samples in distilled water (1:10 w/v) overnight and then filtering them through Whatman No. 1 filter paper; they were then kept in a refrigerator until ready for use. In one case, an aqueous extract of pericarps and seeds were made separately by soaking the samples in distilled water (1:20 w/v) overnight and then used for a germination assay.

Seeds used for the germination assay were variety 569H3 (monogerm) grown at the East Farm of Oregon State University. Seeds were leached for 20 hours in a flowing tap-water and dried completely on paper towels at room temperature and retained at least two months prior to use in germination tests. These seeds were designated as treated seeds in the experiments.

Results

Various solvent extracts of sugarbeet fruits were used as media for germinating sugarbeet seeds.

Both treated and untreated (original) seeds were germinated to compare their responses to the different solvent extracts. The treated seeds showed a marked increase in germination and seedling

vigor compared with corresponding untreated seeds regardless of the type of extracts used (see Table 2). In subsequent tests only treated seeds were used in assays.

Table 2. Effects of germination medium on sugarbeet seed germination. (Variety, 569H3-USDA)

Treatment[1] (Germination media)	% Germination	
	Original seed	Treated seed**
Aqueous extract	91.0	97.0
H_2O_2 (3%) extract	91.0	97.5
Aqueous extract decolor.	89.5	96.0
Distilled water (control)	89.5	97.5

[1]Average of four samples, 50 seeds per sample.
**Significantly higher at 1% level.

As shown in Tables 3, 4 and 5 the water soluble portion of the extract is the most inhibitory, both in terms of germination and seedling growth. These data indicate that the active inhibitory substances are water-soluble compounds with stable chemical characteristics.

Table 3. Influence of several solvent extracts of sugarbeet fruit upon the germination of treated monogerm variety 569H3.

Treatment[1] (Germination media)	% Germination
95% EtOH extract	92.7
MeOH extract	92.0
Acetone extract	88.0*
Ether extract	88.7*
Distilled water extract (1:10)	84.0**
Distilled water (control)	95.3

[1]Average of three samples, 50 seeds per sample.
*Significantly lower at 10% level.
**Significantly lower at 1% level.

Table 4. Effects of flavonoids from sugarbeet fruit on seed germination. (Variety 569H3)

Treatment[1] (Germination media)	% Germination
Ether soluble portion	99.3
Ether insoluble portion (or only water soluble portion)	90.3*
Old aqueous extract (1:10)	97.3
Fresh aqueous extract (1:10)	97.3
Distilled water (control)	98.0

[1]Average of three samples, 50 seeds per sample.
*Significantly lower at 5% level.

Table 5. Effects of aqueous extracts of pericarp and sugarbeet seed on the germination of sugarbeet seed. (Variety 569H3)

Treatment[1] (Germination media)	Sample	% Germination	Seedling[2] length (cm)
Aqueous extract of pericarp (1:20 w/v)	Treated seed	98.7	3.03
Aqueous extract of seed (1:20 w/v)	"	95.3	6.57
Distilled water (control)	"	96.0	7.05
Distilled water	Original seed	80.0**	1.14

[1]Average of three samples, 50 seeds per sample.

[2]Average of 60 seedlings per treatment.

**Significantly lower at 1% level.

Experiment 3. Qualitative and Quantitative Determination of the Chemical Compounds Present in the Aqueous Extract of Sugarbeet Fruit

This experiment was designed to study the type of chemical inhibitory substances present in water extracts of the sugarbeet fruit. Both qualitative and quantitative examinations were conducted for phenolic compounds and oxalates. Comparative examinations of phenolic and oxalic acids in sugarbeet fruits of two different varieties were also made. One variety, AH-1, is a normal commercial variety and the other variety, 4426, is a low germinating sample (58% germination).

Solutions of synthetic phenolic compounds were also used in the sugarbeet germination assay tests to evaluate their significance as inhibitors.

Methods and Materials

Extraction Procedure. Procedure 1: Sugarbeet seeds were ground in a mortar and the resulting powder was extracted by shaking with water (1 gm/10 ml H_2O) for over six hours at room temperature. The resulting solution was acidified and extracted with ether.

Procedure 2: The ground samples of sugarbeet seed were extracted exhaustively with boiling 95% ethanol. The solvent was removed under reduced pressure in a rotary evaporator. The residue was mixed with boiling water and passed through filter paper. The filtrate was acidified and continuously extracted with ether for over ten hours.

Chromatographic Method. The extracts from above were dissolved in a ml of hot 95% ethanol, and applied to large sheets of Whatman No. 1 chromatography grade paper. The chromatographic method used was essentially that of Bohm and Towers (7).

Qualitative Determination. The extract concentrate was examined on paper chromatograms using the descending technique with a mixed solvent, n-butanol; acetic acid; water (6: 6: 3 v/v, BAW), and benzene; acetic acid; water (6: 7: 3 v/v) solvent.

Both one-dimensional and two-dimensional chromatography were used in identification of the unknown compounds. Rf values of the unknown spots of the extracts were compared with the results of co-chromatography of the known chemical inhibitors for final identification. The remaining extracts were used for testing the inhibitory effects upon treated seed.[1]

Quantitative Determination. The total oxalate in sugarbeet fruit, was determined by: 1) extracting the oxalate with 30% hydrochloric acid; 2) precipitating as calcium oxalate from the deproteinized extract; 3) dissolving the precipitate in sulfuric acid; 4) subsequently titrating with potassium permanganate.

Soluble oxalate was removed from the seed with aqueous extraction. The later stages of the determination were carried out on a semi-micro scale, so that a number of determinations could be made simultaneously. The detailed procedures were similar to the methods used by Baker (3) and Zarembski et al. (54).

Quantitative determination of phenolic compounds was conducted by preparing test solutions containing 1 mg of known compounds per ml as standards. Spots of various concentration of these compounds were prepared by repeated addition of drops of the standard solution on filter paper. The diazo-spray reagent was prepared by

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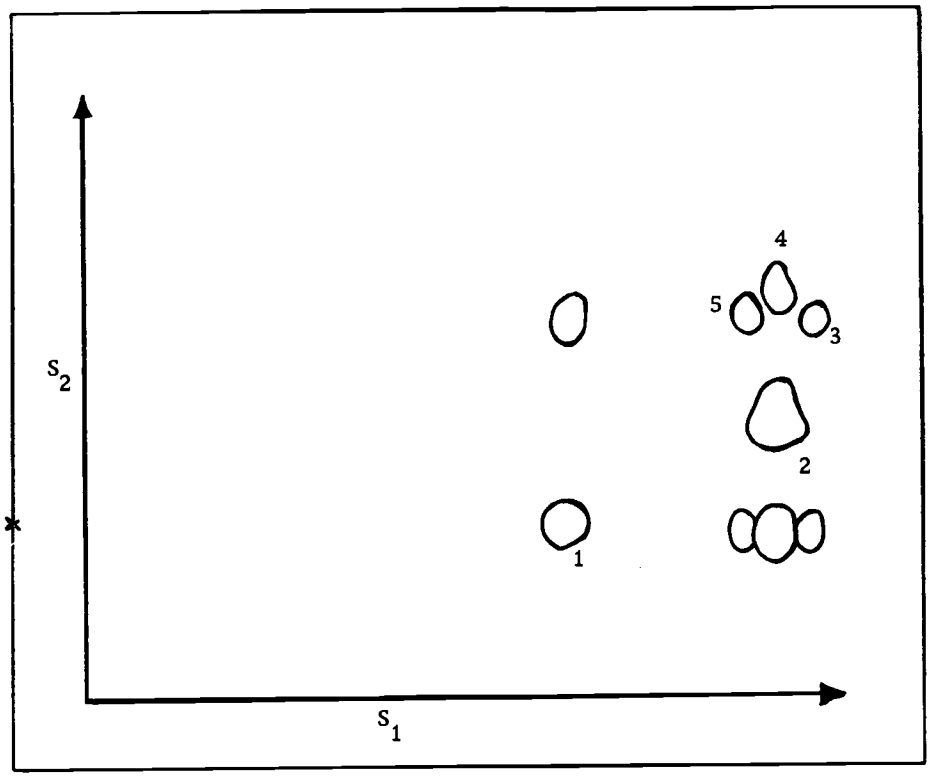
[1] Refer to Experiment 2, Methods and Materials.

dissolving 0.2 gm of Fast Scarlet R salt (commercial name of 2-amino-4-nitroanisole) in 30 ml of methanol. The paper was first sprayed with the above reagent and allowed to dry for spot development, after which, it was sprayed with a 0.1N sodium hydroxide solution. Unknown spots were also examined under ultra-violet light for quantitative estimation along with the compounds of known concentration. The maps showing relative position of the known and unknown compounds were given in Figures 2 and 3.

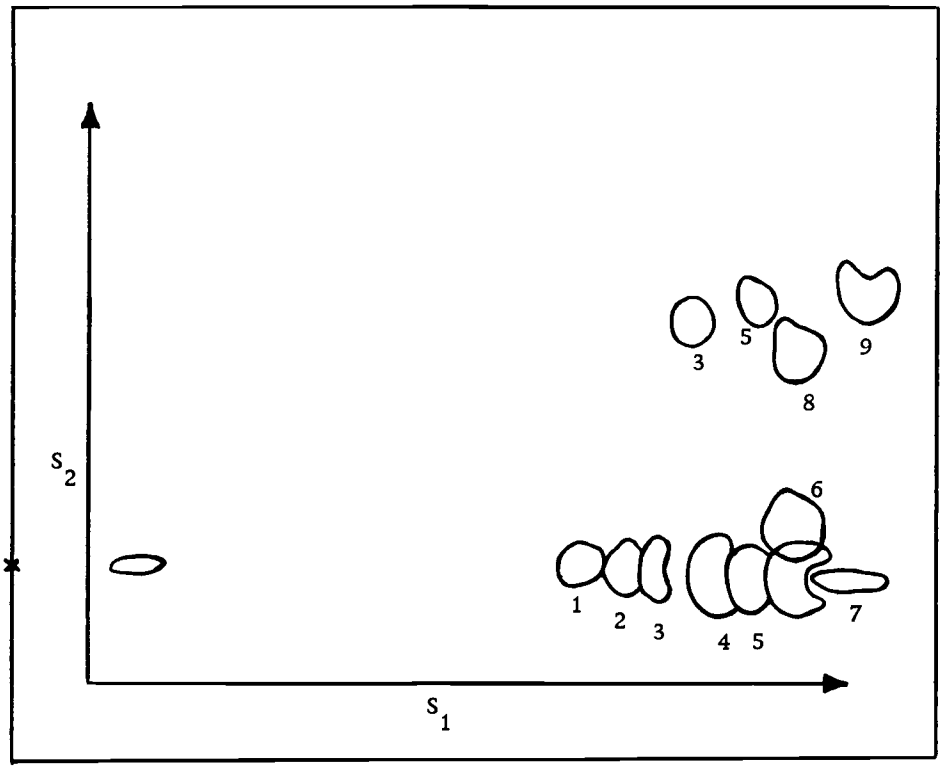
Quantitative determination of oxalic acid was very similar to those used by Baker (3) and Zarembski et al. (54) with slight modification in acid concentration used for insoluble oxalate extraction. Briefly, it involved extraction with 30% hydrochloric acid for total oxalates, precipitation as calcium oxalate from the deproteinized extract and subsequent titration with potassium permanganate. Soluble oxalates were determined in a similar manner, in aqueous extract. The later stages of the determination are carried out on a semi-micro scale, so that a number of determinations can be made simultaneously.

Sugarbeet Seeds Used for Germination. Treated seeds of variety 569H3 were used for germination experiments. The seeds were harvested on August 20, 1967 and were leached in flowing tapwater (with a flow rate of two liters per minute) for 20 hours and dried completely before germination for at least one month at room



Spot	Compound	Rf value S_1	Rf value S_2
1	Gallic acid	.62	.61
2	Ferulic acid	.84	.56
3	Vanillic acid	.88	.72
4	t-Cinnamic acid	.85	.75
5	p-OH-Benzoic acid	.82	.69

Figure 2. Two-dimensional chromatogram. Map showing the relative positions on a two directional chromatogram of major phenolic compounds known to be present in sugarbeet seeds. S_1 represents Benzene:Acetic acid:Water (6:7:3); S_2 represents 2% aqueous formic acid.



	Spot #								
Rf value	1	2	3	4	5	6	7	8	9
S_1	.61	.65	.69	.76	.81	.85	.91	.85	.90
S_2	-	-	.64	-	.70	.40	.30	.68	.78

Figure 3. Two-dimensional chromatogram of aqueous sugarbeet fruit extract. Map showing the relative positions on a two-dimensional chromatogram of major phenolic compounds in sugarbeet extract including unidentified substances. S_1 represents Benzene:Acetic acid:H_2O (6:7:3); S_2 represents 2% aqueous formic acid.

temperature.

Results

Identification of the unknown phenolic compounds in the aqueous extract of sugarbeet seeds and fruits was accomplished by paper chromatographic techniques. Rf values for known compounds were compared with unknown spots under u.v. light; the spots were then developed by diazo-sprays. The results on the phenolic compound investigations are summarized in Tables 6, 7 and 8. Two dimensional chromatograms of known and unknown compounds are shown in Figures 2 and 3.

Table 6. Rf values and color reactions of phenolic compounds.

Compounds	Rf[1]	Fluorescence	Diaz.	Rf[2]	Fluorescence	Diaz.
t-Cinnamic acid (p-Coumaric)	.91	Blue-violet	-	.88	Blue-black	-
Vanillic acid	.87	Grey	Orange	.84	Grey	Orange
p-OH-benzoic acid	.86	Grey	Blue-black	.83	Grey	Blue-black
Ferulic acid	.85	Blue	Blue-black	.85	Blue	Blue-black
Gallic acid	.63	Grey	-	.60	Grey	-

[1] n-Butanol; acetic acid; water solvent.

[2] Benzene; acetic acid; water solvent.

Table 7. Comparison of Rf values of phenolic substances including unknowns from extracts of two different sugarbeet varieties.

Variety	Rf value	Spot No. 1	2	3	4	5	6	7	8	9
AH-1	Rf^1	.93	.88	.86	.83	.78	.68	.61	.39	.27
4426	Rf^1	.94	.89	.85	.82	.79	.68	.60	.40	.28
AH-1	Rf^2	.95	.86	.82	.76	.71	.64			
4426	Rf^2	.94	.87	.82	.77	.72	-			

[1] n-Butanol; acetic acid; water solvent.

[2] Benzene; acetic acid; water solvent.

Table 8. Quantitative estimation of ferulic acid present in the aqueous extract of two sugarbeet varieties; variety AH-1 and 4426.

Variety	Component: Seed (mg)	% by weight	Pericarp (mg)	% by weight	Fruit (mg)	% by weight
AH-1	1.2	.12	14	1.4	12	1.2
4426	3.8	.38	30	3.0	26	2.6

The spots shown by chromatography were almost identical regardless of the seed source that was extracted. This suggested that the chemical components of all sugarbeet varieties were similar.

The data shown in Table 8 indicate that ferulic acid content of both pericarp and caryopsis in variety AH-1 is considerably less than that in variety 4426. This may indicate that ferulic acid is responsible for low germination in variety 4426. The concentration difference within the caryopsis of 4426 and AH-1 was much greater than the difference in the pericarp of the two varieties. This may indicate that the inhibitors present in the caryopsis exert more influence on dormancy control than inhibitors in the pericarp.

Difficulties encountered in the identification of unknown substances were due to overlapping of unknown spots. In some cases the separation of the spots was possible by two dimensional chromatography. This experiment was repeated three times before relatively good separations were achieved. The Rf values of the known and unknown spots were determined by taking the average of three or four readings.

Quantitative analysis data of oxalic acid and its salt indicated that every variety of the sugarbeet fruit contained a considerable amount of these substances, but the concentrations varied from one variety to another (see Table 12).

Unexpectedly, the level of the oxalate concentration in the

variety of 4426 was the lowest of the varieties tested as shown in Table 12. This data suggest that oxalic acid or oxalates are not directly related to dormancy control; however, oxalates adversely affect the seedling growth as shown in Tables 9 and 10.

Table 9. Effects of oxalic acid and its potassium salt solution on the germination of monogerm sugarbeet seed.

Sample	Media	Concentration	% of Germ.	Seedling length (cm)	Remarks
Original seed	Dist. water	-	72.6	1.3	
Treated seed (control)	Dist. water	-	96.0	7.3	
Treated seed	Oxalic acid	0.01M	95.5	1.3	All of the seedlings showed identical symptom of root tip blackening except control.
Treated seed	K-oxalate	0.01M	96.0	0.9	
Treated seed	Oxalic acid	0.1M	90.6	0.7	
Treated seed	K-oxalate	0.1M	92.0	0.8	

Experiment 4. Examination of Limed and Unlimed Sugarbeet Seeds to Determine their Oxalate Content

In this experiment, germination and oxalate content of seeds produced on limed and unlimed soil were compared.

Methods and Materials

Extraction Technique. The methods of extraction employed

Table 10. Effects on germination and seedling vigor of pure chemical solutions known to be present in sugarbeet seed as germination inhibitors.

Compound	Concentration	% of Germination[1]	Seedling length (cm)[2]	Remarks
Distilled water (control)	-	97.5	3.87	Normal seedling
p-OH-benzoic acid	0.01M	98.5	3.22	Weaker than control
Ferulic acid	"	95.5	1.74	Showed abnormal growth, later root tip turned brown
Gallic acid	"	100.0	3.14	Seedlings were more vigorous than control
t-Cinnamic acid	"	96.0	1.94	Symptoms similar to those of ferulic acid
Vanillic acid	"	98.0	3.44	Similar to ferulic at early stage
Oxalic acid	"	96.5	1.26	Root tips turned brown and growth was retarded
K-oxalate	"	96.6	1.28	Identical symptom to oxalic acid
Crude extract	1:10	97.0	2.19	Similar symptoms to that of oxalate

[1] Results are the average of four samples, and 50 seeds per sample.

[2] Results are the average of 40 seedlings per treatment.

Table 11. Recoveries of oxalate added to samples of sugarbeet seed.

	Oxalic acid equivalent of Na-oxalate (mg)	Oxalic acid found in added sample (mg)	Oxalic acid found in control (mg)	Recovery of oxalic acid (mg)	% of Recovery
R_1	.672	4.275	4.600	.675	100.44
R_2	1.076	6.840	5.808	1.032	95.91
R_3	.874	5.499	4.670	.829	94.85

Average recovery is 97.67%.

Table 12. Oxalate content in seed lots of some commercial varieties of sugarbeet fruits.

Description	Variety			
	AH-1 %	USH-9B %	569H3 %	4426 %
Total oxalate	1.4485	1.5353	1.5096	1.2103
Soluble oxalate	.6526	.5906	.6990	.4942
Insoluble oxalate	.7959	.9447	.8106	.7161
Pre-soaking germination	89.4	84.6	94.5	58.6

in this work were the same as those used in experiment 3-E and quantitative determineation of oxalates was similar to that used in previous work.

Seed Sample. Limed (L-2-12) and unlimed (U-2-12) monogerm sugarbeet seeds were available through the courtesy of the seed laboratory at Oregon State University.

Results

Quantitative determination of oxalates in aqueous extracts of limed and unlimed sugarbeet fruit was made to compare the level of oxalates (soluble and insoluble oxalates) between the two samples.

Results of the experiment are summarized in Table 13. Although the unlimed sample contained a slightly higher amount of oxalates, overall germination of unlimed seeds is slightly higher than that of limed seeds. The difference of oxalate content existed in both seed lots would seem too small to be a factor in dormancy control. The insoluble oxalate content shown in Table 13 is somewhat less than expected and this low value might have resulted from incomplete extraction.

Table 13. Oxalate contents of sugarbeet seed produced on limed and unlimed soil.

Content	Percentage[2]	
	Sample	
	Limed (L-2-12)	Unlimed (U-2-12)
Total oxalate	1.4491	1.5474
Soluble oxalate	.6898	.7470
Insoluble oxalate	.7593	.8004
% Germination[1]	86.6	94.6

[1]Average of three samples, 50 seeds per sample.

[2]Average of three readings.

Experiment 5. Effects of Maturity on Sugarbeet Seed Germination

These experiments were conducted to determine if seed maturity is associated with low germination, and to examine the effects of ageing on germination.

Methods and Materials

The seed samples were collected from the East Farm of Oregon Agricultural Experiment Station, Corvallis at regular intervals starting from the later part of July through September in 1967 and 1968. Random samples of 10 to 15 plants were harvested for each collection, brought to the laboratory, and dried at room temperature. Dried

samples were cleaned and kept separately in bags until used for germination.

Samples harvested in 1967 were germinated at three time intervals during two year period.

Results

The results are summarized in Tables 14, 15 and 16 and in Figure 4. The sample harvested in 1967 indicated some relationship between harvesting date and germination. This type of pattern is lacking in the 1968 samples; the unseasonal rainfall in 1968 might have been responsible for different results in the two years.

The germination experiments with the 1968 seed lots that were dissected are presented in Table 15. The percentage of ungerminated seeds is much greater in the small seeds. In early harvest samples, a relatively small portion of the seed lots comprises empties; however empty seeds appeared to be a major factor causing germination failure in later harvests. Possibly empty seeds resulted from poor pollination, abnormal pollen due to rainy conditions, or by lygus bug.

The data shown in Table 16 reveals that ageing did not markedly improve the germination potential of a given sample. The data also suggest that inhibitors present in the seedball are stable over time.

Table 14. Effect of maturity on the germination of monogerm sugarbeet seed.

Harvest date in 1967	% Germination[1]	Harvest date in 1968	% Germination[2]
July 31	96.0	July 29	80.0
August 7	94.0	August 5	82.4
August 16	94.8	August 12	83.0
August 23	84.3	August 22[3]	87.0
September 5	89.3	September 14[3]	80.3

[1]Average of four samples, 100 seeds per sample.

[2]Average of three samples, 50 seeds per sample.

[3]Samples collected after heavy rain.

Table 15. Effects of harvesting date upon the germination of different size classes of sugarbeet seed harvested in 1968.

	Date of harvest					
	7-24	7-29	8-5	8-12	8-22[1]	9-14[1]
Large seed[2]						
% Germination	51.33	82.67	90.00	90.00	86.67	86.00
% Ungerminated	48.67	17.33	10.00	10.00	13.33	14.00
Empty	.67	1.33	1.33	.67	3.33	6.33
% Abnormal	4.67	.67	3.33	2.00	6.67	4.00
Normal	43.33	15.33	5.34	7.33	3.33	3.67
Small seed[3]						
% Germination	70.00[4]	77.33	75.33	76.00	87.33	74.67
% Ungerminated	30.00	22.67	24.67	24.00	12.67	25.33
Empty	5.33	.67	10.67	9.34	9.34	21.33
% Abnormal	10.00	6.67	3.33	7.33	2.00	2.67
Normal	14.67	15.33	10.67	7.33	1.33	1.33
Average						
% Germination	60.67	80.00	82.67	83.00	87.00	80.33
% Ungerminated	39.33	20.00	17.33	17.00	13.00	19.67
Empty	3.00	1.00	6.00	5.00	6.33	13.84
% Abnormal	7.00	3.67	3.33	4.67	4.34	3.33
Normal	29.00	15.33	8.00	7.33	2.33	2.50

[1]Samples collected after rains.

[2]Seeds retained on No. 9 screen.

[3]Seeds passed through No. 9 screen.

[4]Ungerminated seeds were treated for 12 hours and then regerminated. The germinated portion was added to the total germination.

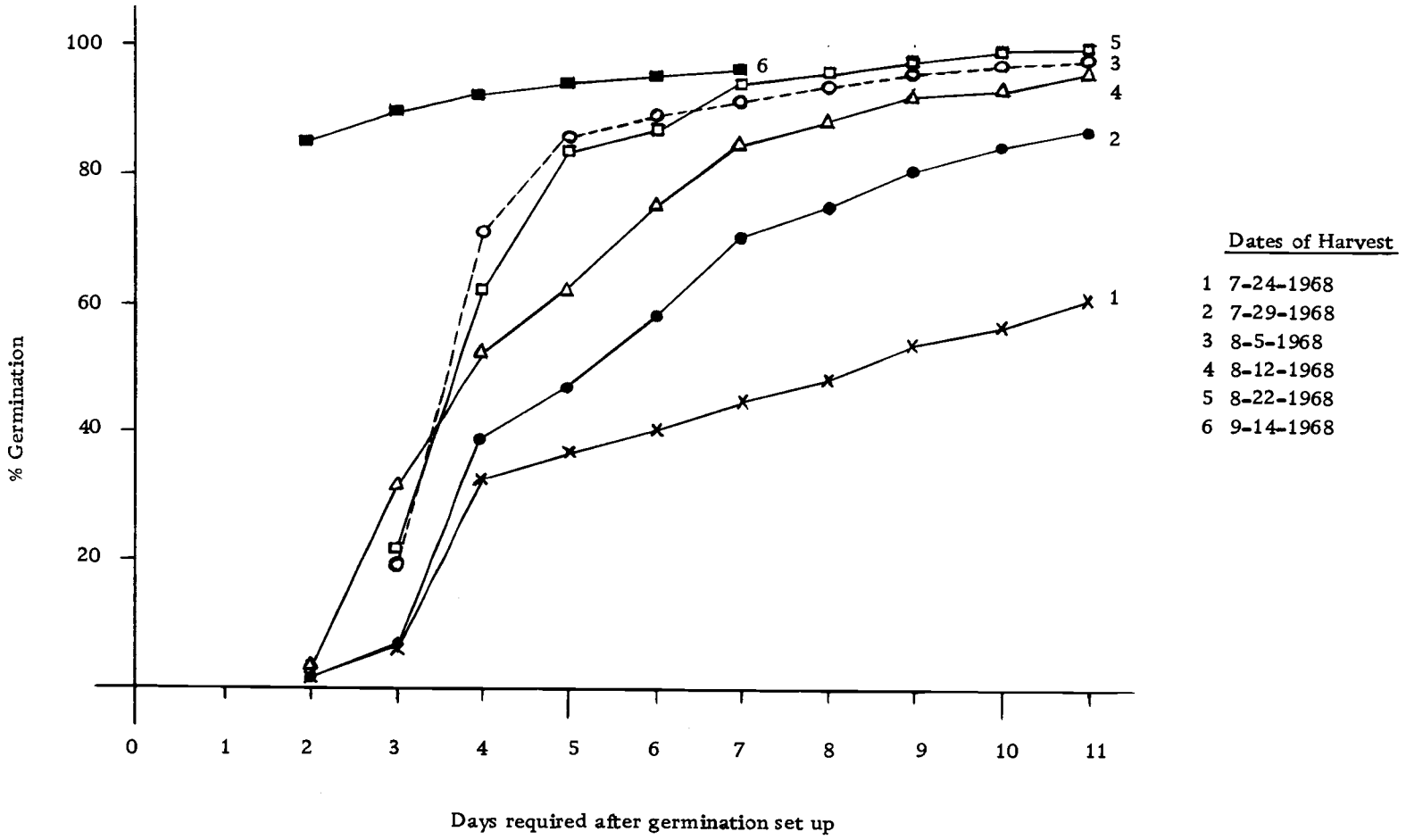


Figure 4. Cumulative germination results, variety 569H3 in 1968. Harvested at different times.

Table 16. Influence of seed age upon germination percentage. (Variety 569H3)

Sample No.	Date of harvest	Date of germination: Jan. 8, 1968 % Germination[1]	Nov. 19, 1968 % Germination[2]	May 10, 1969 % Germination[2]
1	7-31-67	96.0	98.7	99.0
2	8-7-67	94.0	92.0	94.0
3	8-16-67	94.8	95.3	93.0
4	8-23-67	84.3	81.3	78.0
5	9-5-67	89.3	89.3	89.0

[1]Average of three samples, 100 seeds per sample.

[2]Average of three samples, 50 seeds per sample.

Experiment 6. Effects of Leaching, Soaking and Drying on Sugarbeet Seed Germination

The experiment to be described was designed to test the effects of water leaching, soaking and drying after leaching on subsequent germination.

Methods and Materials

Seed samples were leached in flowing tap-water for a designated period of time under submerged conditions. The leached seeds were dried on paper towels at room temperature. The dried seeds were kept in the laboratory in a paper sack until germination.

The seeds which were dried after leaching are designated as "Treated seed" to differentiate them from samples which have been leached only. The seed germination and seedling vigor were evaluated as described in General Methods and Materials.

Flow rate of tap-water was adjusted to approximately 2.5 liters per minute. Water temperature recorded at intervals of six hours was 12° ($\pm$ 4°) C during the leaching period.

Monogerm sugarbeet varieties used for germination in the experiments involved all the commercial varieties on hand including 569H3-USDA. Variety 4426 was also used to evaluate the effects of various pre-germination treatments on its germination.

Results

The experimental data shown in Tables 18, 19, 20, 21 and 22 and Figures 5 and 6 indicate that there are differences between various leaching and drying procedures on subsequent seed germination.

The marked increase of germination from treated samples (completely dried seeds after water leaching) shown in Tables 17, 18, 20 and 21 and Figure 6 was not merely due to leaching out inhibitors to the point where they are no longer effective to control dormancy. As shown in Table 20 simple leaching did not improve germination. The necessary requirement of several days or longer drying period after leaching may suggest a complex physiological change occurred

Table 17. Comparison of leaching vs. soaking effects on the germination of monogerm sugarbeet seeds.

Treatment[1]	% Germination[2]	
	Sample A	Sample B
Seeds leached with flowing water	97.4**	97.4**
Seeds leached with limited water (1:1)	78.0	78.6
Seeds with no treatment	73.4	71.4

[1]All samples dried for two weeks after water treatment.

[2]Average of three samples, 50 seeds per sample.

**Significantly higher at 1% level.

Table 18. Effects of leaching treatment upon the germination of monogerm sugarbeet varieties.

Treatment[1]	Variety				Days required
	AH-1 %	HH-5 %	USH-9B %	569H3 %	
Original seed	89.4	80.6	84.6	89.5	14
Treated seed**	96.0	90.6	98.6	97.5	7

[1]Average of three samples, 50 seeds per sample.

**Significantly higher at 5% level.

Table 19. Comparison of treated and untreated samples of variety 569H3 harvested at different times.

Harvest date	Treatment			
	Original seed		Treated seed	
	% Germination[1]	Length of seedlings[2] (cm)	% Germination[1]	Length of seedlings[2] (cm)
7-31-67	96.0	.98	98.0	4.93
8-7-67	94.0	1.80	97.0	4.54
8-16-67	94.8	1.48	96.3	4.12
8-23-67	84.8	1.79	94.8	5.36
9-5-67	89.3	1.42	92.8	4.97

[1] Results of eight day-germination.

[2] Results of six day-germination. Average of 40 seedlings from each treatment.

Table 20. Effects of leaching and drying on the germination of sugar-beet seeds (variety 569H3) harvested from a single plant.

Treatment[1] of sample	% Germination	% Ungermination		Adjusted % germ.
		Undeveloped	Normal	
Original seed	62.0	9.33	28.67	68.25
15 hours leached and 1 day dried	64.0	9.33	27.67	70.56
15 hours treated (dried for 3 weeks)	86.7	12.00	1.30	98.67**

[1] Average of three samples, 50 seeds per sample.

**Significantly higher at 0.1% level.

Table 21. Effect of drying period upon germination of leached sample.[1]

Date of harvest	Days after leaching							
	1		30		360		600	
	% Germ.	Days req.	% Germ.	Days req.	% Germ.	Days req.	% Germ.	Days req.
7-31-67	96.5	8	98.0	8	98.7	5	98.0	5
8-7-67	89.8	"	97.0	"	97.3	"	99.0	"
8-16-67	88.5	"	96.3	"	96.0	"	94.0	"
8-23-67	71.3	"	94.8	"	93.0	"	94.0	"
9-5-67	71.5	"	92.8	"	94.7	"	98.0	"
Date of germination	11-24-67		12-24-67		11-19-68		5-10-69	

Table 22. Comparison of germination after several pre-germination treatments for sugarbeet variety 4426.

Percentage	Treatment					
	1 hour soak in in clorax	Standard method	H_2O_2 method	1 hour soak in 1.5% clorax	48 hrs. ** treated seed	Average
Total % germination[1]	33.33	42.67	43.33	49.33	79.33	49.60
% of un-germination	66.67	57.33	56.67	50.67	20.67	50.40
% Empty	4.67	4.00	5.33	8.00	4.67	5.33
% Abnormal	19.33	10.66	15.67	17.33	14.00	15.40
% Normal	42.67	42.67	35.67	25.34	2.00	29.67

[1] Average of three samples, 50 seeds per sample.

**Significantly higher at 1% level.

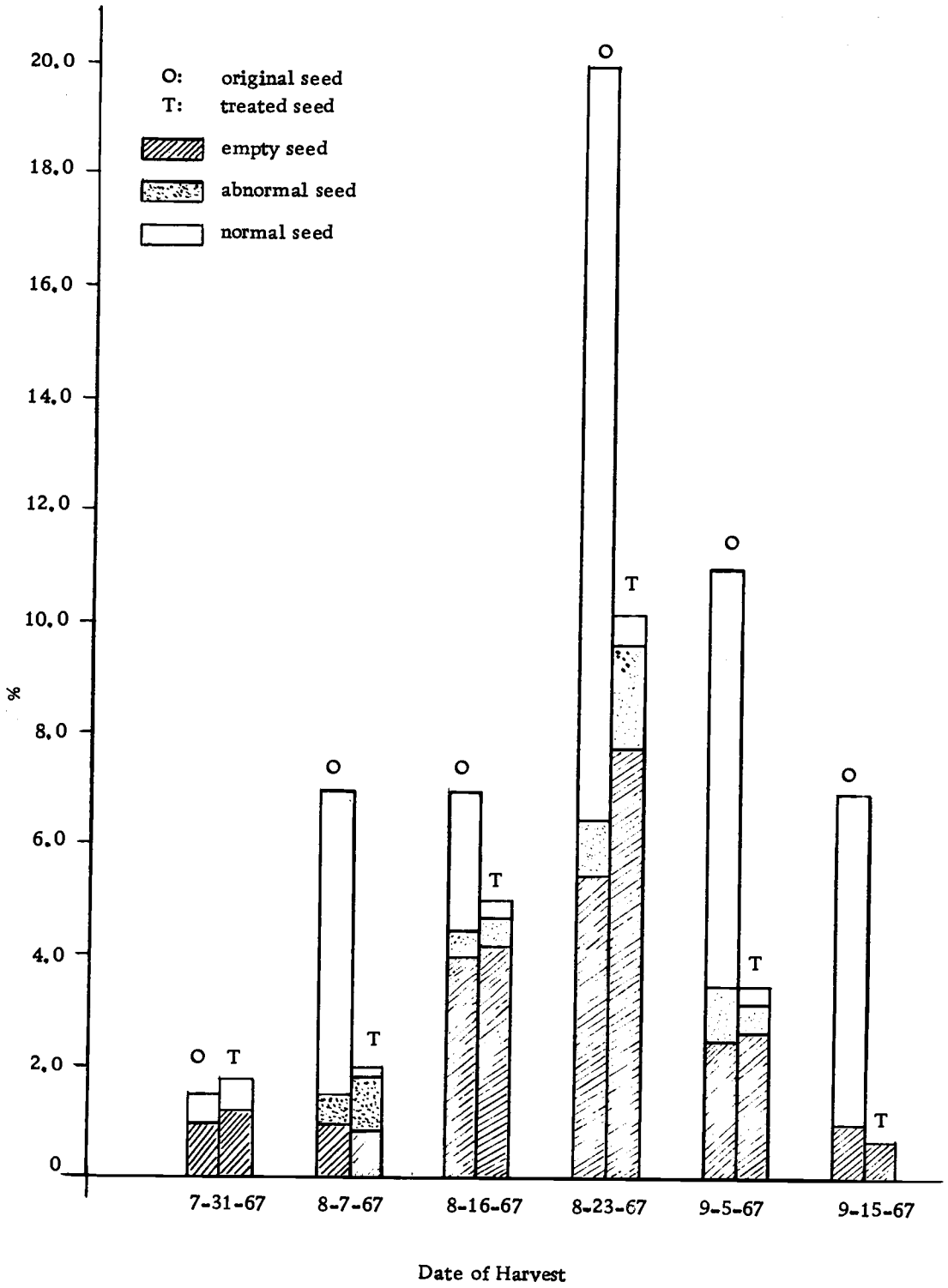


Figure 5. Analysis of ungerminated seeds by cutting method; untreated samples vs. treated samples.

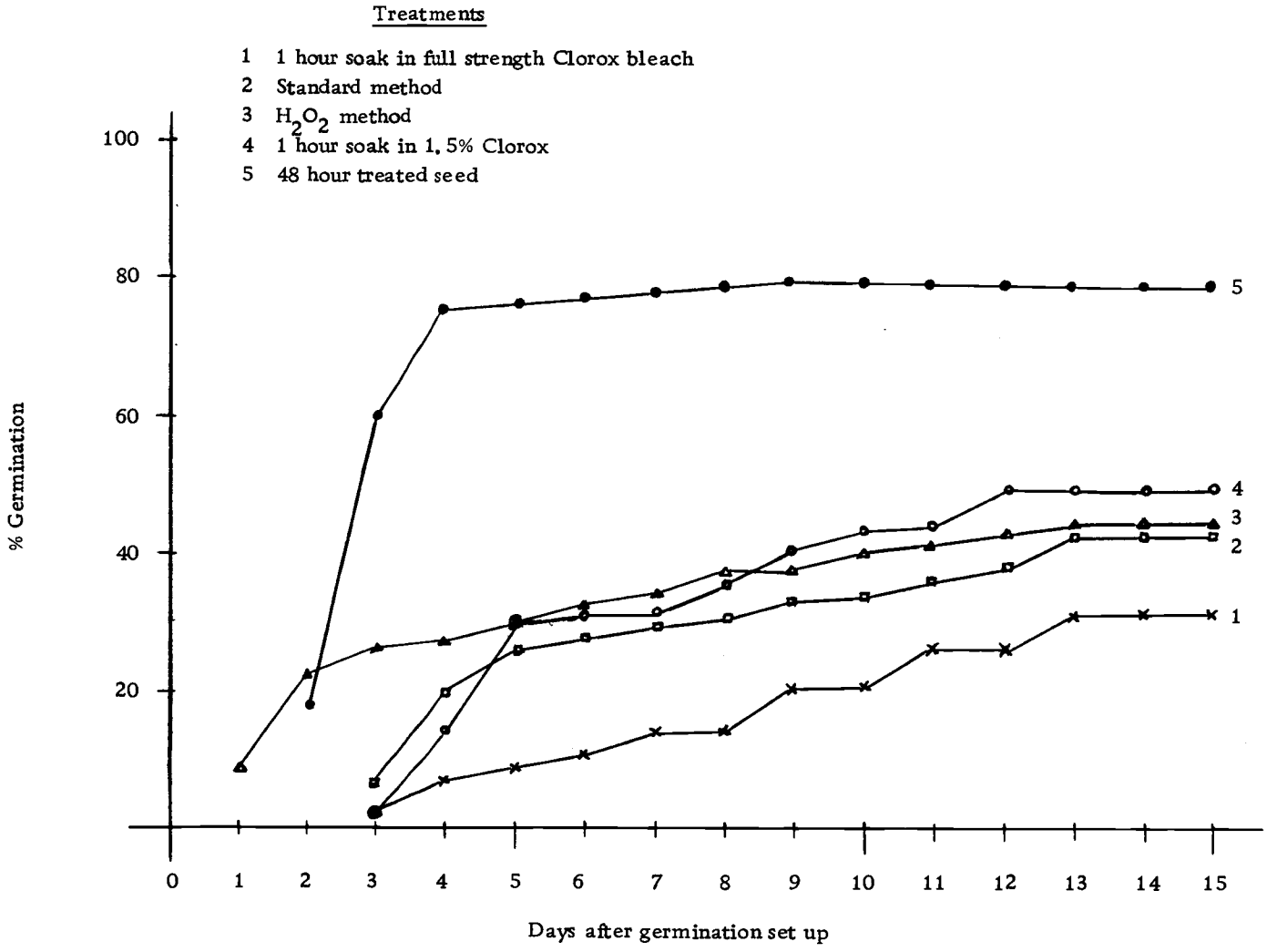


Figure 6. Cumulative germination percentage of sample from variety 4426, a low germinating sample, after various pre-germination treatment.

in the seeds during and after leaching.

The increased germination and vigor of the seedling from treated samples may indicate a shift of a promotor-inhibitor complex in favor of promotors.

Experiment 7. Studies on Leaching Time and Its Effect on Germination and Chemical Composition

This experiment was designed to determine the optimum leaching period in connection with germination potential of the leached seeds, to make a quantitative estimation of substance loss by leaching, and finally the changes of some major compounds such as ferulic and oxalic acids contents present in leached samples by using analytical techniques.

Methods and Materials

Leaching Procedure. Seeds of variety AH-1 were selected and grouped in three classes i.e. small, medium and large.[2] An equal number of seeds were selected from each class per treatment and bagged together. The bagged samples were put in one liter beaker and placed under flowing tap-water with a flow rate of about two liters per minute. The bags were taken out from the beaker at regular

[2] Refer to Experiment 1. Methods and Materials for description of the different classes.

intervals and dried on paper towels.

Analytical Methods. The methods used for oxalate determination of the leached seeds were identical to those described in Experiment 3. The quantitative determination of ferulic acid was based on paper chromatographic technique similar to that used by Schroeder (35).

A test solution containing 1 mg of ferulic acid per ml was prepared as a standard. Spots of various concentrations were prepared by repeated addition of drops of this standard solution on filter paper. Paper chromatograms of unknown concentration of ferulic acids from a known amount of extract were also prepared. Both sets of chromatograms were compared by ultraviolet light and by color development with diazo reagent, to estimate the concentrations of unknowns.

Seed Samples. Only one variety, AH-1, was used in this experiment in order to avoid variations which might result from varietal differences.

Results

The data for these experiments are summarized in Table 23, 24 and 25 and Figures 7, 8, 9 and 10. A gradual germination improvement, with increased leaching time is shown by the data in Table 23. The optimum leaching time for this particular sample was 12-20 hours. Even though the seeds subjected to 32 hours of leaching

Table 23. Effects of leaching period on the germination of sugarbeet seed. (Variety AH-1)

Treatment No.	Leaching period (hour)	% Germination	% Ungermination seed			% of germination after adjust	Seedling length (cm)
			Total	Undeveloped	Normal		
1	0	94.0	6.0	1.3	4.7	95.3	1.48
2	1/2	90.0	10.0	8.3	1.7	98.2	3.09
3	1	90.2	9.8	8.3	1.5	98.4	3.15
4	4	90.0	10.0	9.2	0.8	99.1	4.15
5	8	95.8	4.2	2.5	1.7	98.3	4.29
6	12	97.2	2.8	2.8	0	100.0	4.32
7	16	90.0	10.0	8.3	1.7	99.1	6.79
8	20	88.3	11.7	11.7	0	100.0	6.75
9	24	94.5	5.5	5.5	0	100.0	6.92
10	28	90.0	10.0	10.0	0	100.0	6.50
11	32	89.2	10.8	10.8	0	100.0	7.14
Average		91.7	8.3	7.2	1.1	98.9	4.96

[1]Results of seven day-germination and the average of three samples, 100 seeds per sample.

Table 24. Oxalate content in leached samples of sugarbeet seed. (Variety AH-1)

	Period of leaching (hours)				
	0	1	4	12	24
Treatment	% by air dry weight[1]				
Total oxalate	1.5057	1.3148	1.1573	1.0677	1.0552
Soluble oxalate	.6798	.4888	.3758	.2904	.2500
Insoluble[2] oxalate	.8259	.8260	.7815	.7773	.8052

[1]Average of three separate readings.

[2]Insoluble oxalates were obtained by subtracting soluble oxalates from total oxalates.

Table 25. Ferulic acid content in leached samples of sugarbeet seed.

Treatment	Loss of weight (mg)	% loss by weight
0 hour	12.0	1.2
1 hour	11.0	1.1
4 hours	6.0	.6
12 hours	4.5	.45
24 hours	3.0	.3

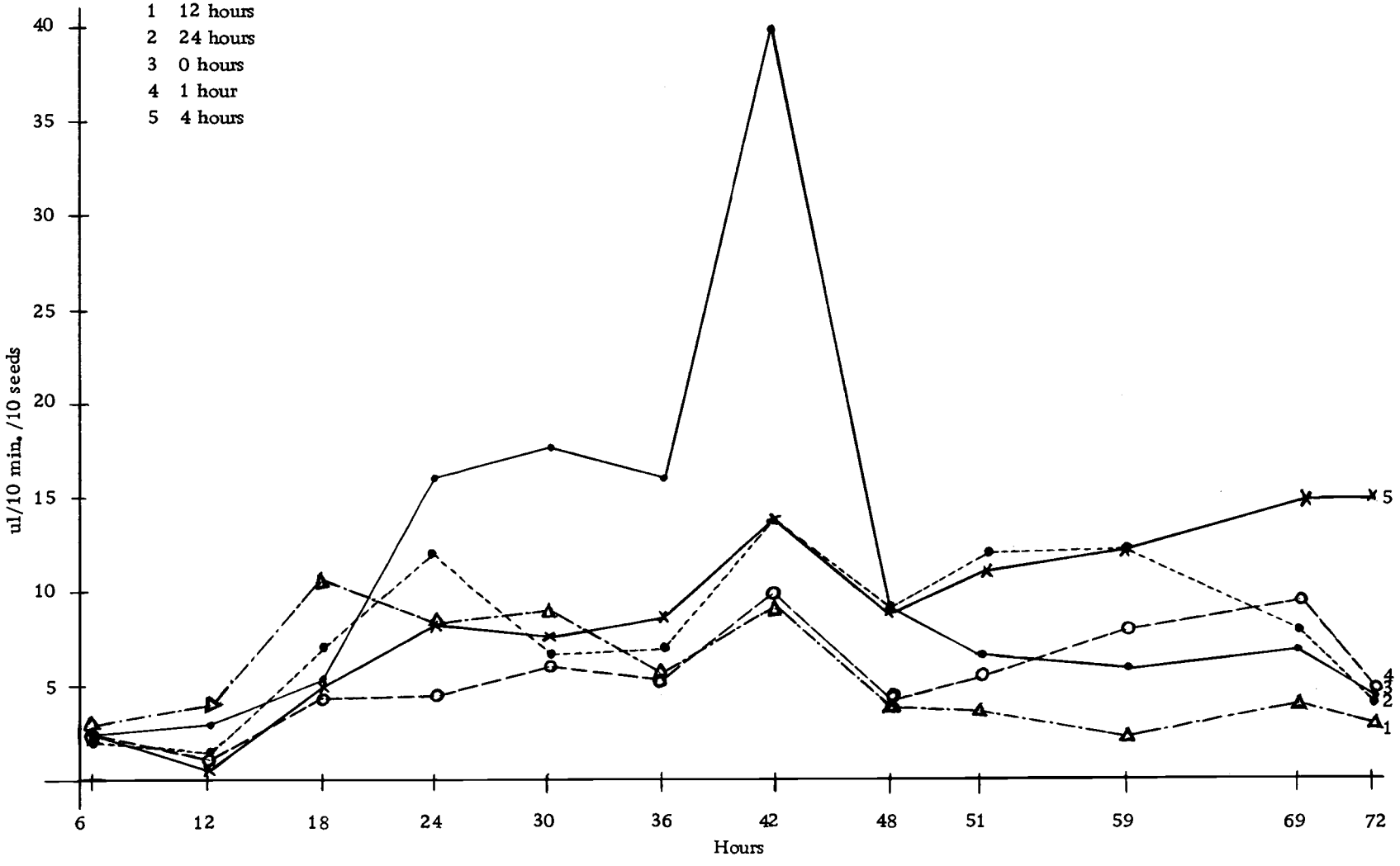


Figure 7. Rate of oxygen consumption by germinating seeds.

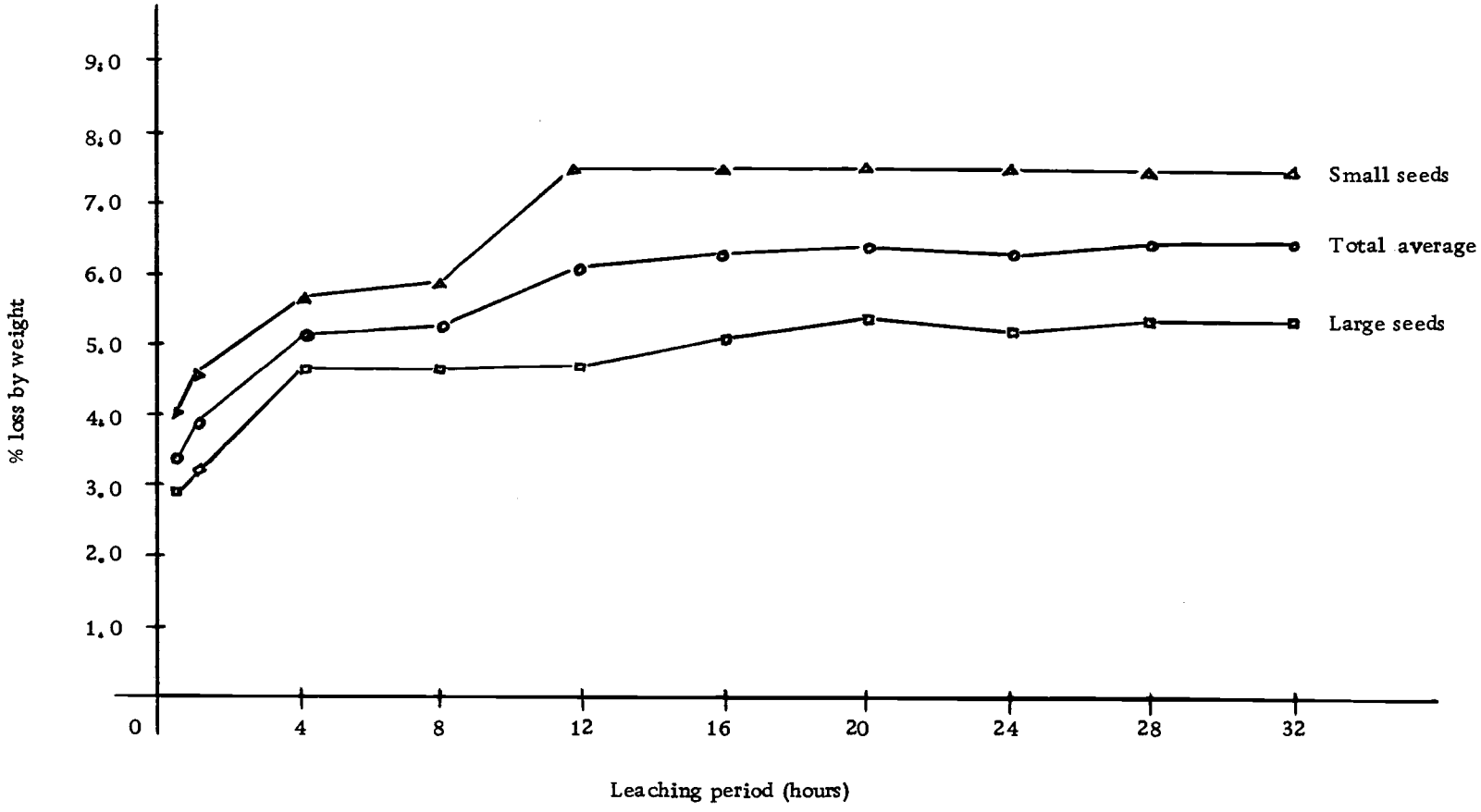


Figure 8. Loss of substances from sugarbeet seed by leaching.

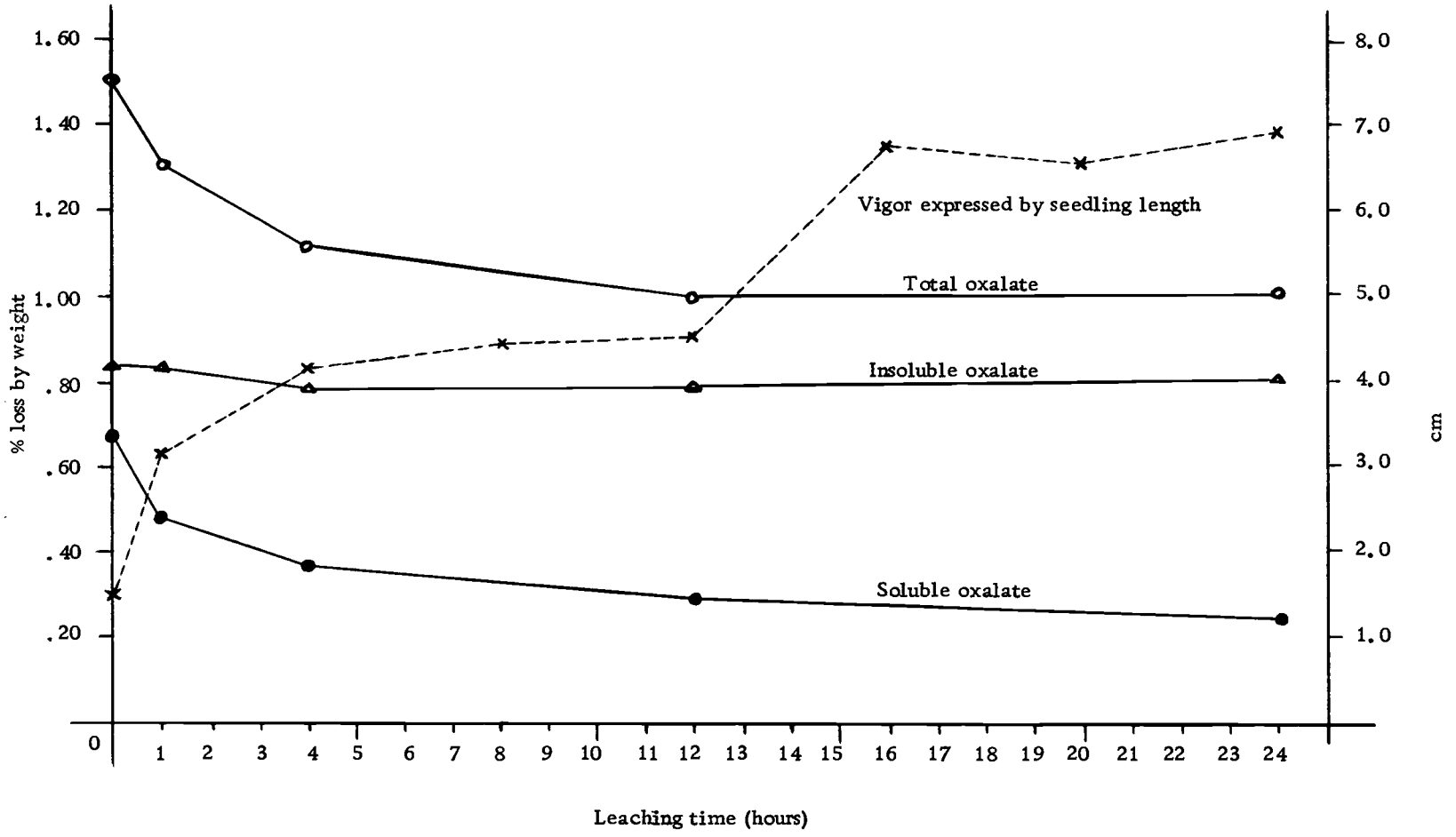


Figure 9. Oxalate content of sugarbeet seedball after leaching treatment.

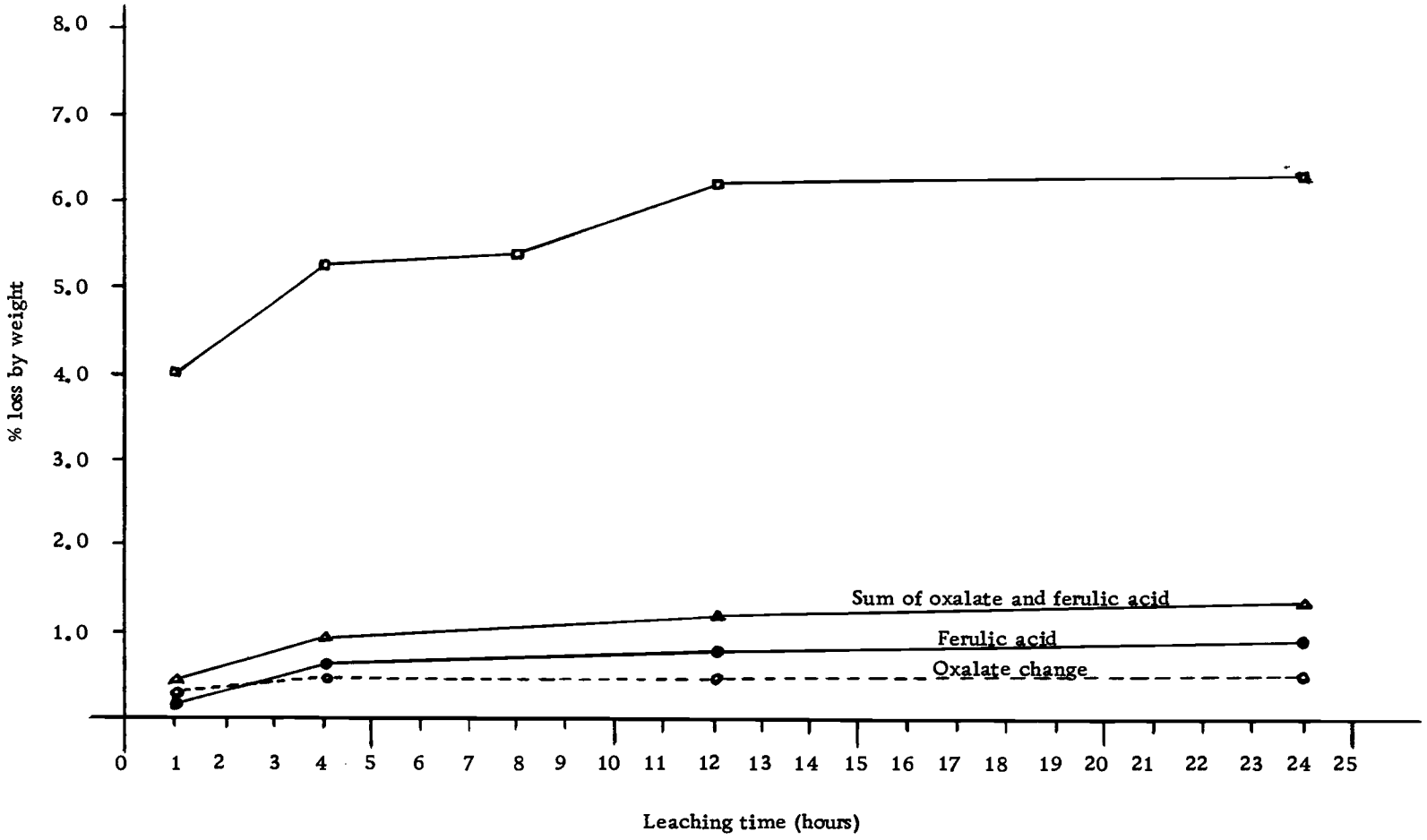


Figure 10. Effect of leaching time on inhibitor content of monogram sugarbeet seedball.

gave the same germination percentage and initial seedling vigor as those of 12-20 hour leaching, the seedling vigor was maintained only for 2-3 days. Apparently long periods of leaching may reduce the overall vigor of the seedlings. The data from the respiration experiments shown in Figure 7 indicate a higher oxygen consumption in the 12 hour leached seeds than any seed lot other than the unleached seeds. The high oxygen consumption by unleached seeds may be due to the presence of inhibitors which are directly involved in oxidation.

The data shown in Tables 24 and 25 are the changes in oxalate and ferulic acid concentration due to various leaching times. In case of oxalates, only the soluble oxalate level decreased with leaching while the insoluble oxalates remained about the same over the extended leaching time. This result might be expected because insoluble forms of oxalate were either Ca or Mg salts of low water solubility. Although the change in soluble oxalate is quite rapid within the first few hours from initial point of leaching, a portion of the soluble oxalate still remained in the fruit after 24 hour leaching.

Similar disappearance phenomena of phenolic substances including ferulic acid were observed. The first 1-2 hours of leaching gave rapid changes in these substances in fruits but this change slows down as leaching time is extended (see Figure 10).

Experiment 8. The Effect of GA on the Germination of Sugarbeet Seed

The following experiments were conducted to investigate the nature of gibberellic acid's effect on germination, and to determine to what extent germination capacity is controlled by a gibberellin-inhibitor complex.

Methods and Materials

Treatment Procedure. The following procedure was used in preparing material for germination tests. Seeds were allowed to imbibe water for two hours and then transferred to a known concentration of gibberellic acid potassium salt solution. After 15 hours of soaking, the seeds were transferred to germinating petri-dishes containing Whatman No. 1 filter papers moistened with distilled water.

Seed Samples Used for Germination. The varieties chosen to compare their responses to GA treatment were USH-9B, a normal commercial variety; and 4426, a poor germinating sample.

Results

The results of this experiment are summarized in Table 26. GA-potassium salt solutions in the germination media did not increase germination potential in either variety.

As the concentration was increased from 50 ppm to 200 ppm

germination was greatly decreased in both varieties. Both treated and original seeds responded the same way toward the concentration increase. As would be expected, the treated seeds of both varieties showed higher germination than the original sample.

Table 26. Effects of gibberellic acid (potassium salt) on the germination of monogerm sugarbeet seed.

Sample variety	% Germination					
	Original seed			Treated seed[1]		
	0 ppm	50 ppm	200 ppm	0 ppm	50 ppm	200 ppm
USH-9B	90.0	90.0	86.0	96.7	92.7	92.0
4426	40.0	64.0	34.0	78.0	75.3	76.6

[1]Original seeds were completely dried after a 24 hour-leaching and were kept in bags at room temperature until ready for germination.

Experiment 9. Effects of Drying Temperature on the Germination of Harvested Sugarbeet Seeds

This experiment was designed to investigate a possible effect of drying temperature after harvest on the germination of monogerm sugarbeet seed.

Methods and Materials

Procedure. Two sugarbeet plants were selected from the experimental farm in August 1967. The selection was based on appearance. Over 70 percent of the seedballs of one plant were

straw-colored, whereas in the other plant less than 40 percent of the fruits were yellow. Each of the plants was divided into two parts by taking off alternate branches starting from the bottom. One group of the branches was dried in a cold room while the other group was dried in 80°F growth chamber. Two months later, seeds from both chambers were germinated without pre-germinating treatment.

Results

The data on the effect of various drying temperature upon germination are briefly summarized in Table 27. By drying the harvested seeds in a high temperature, germination was significantly increased in plant A but not in plant B.

Table 27. Influence of drying temperature following harvest upon the germination of sugarbeet seeds collected from a single plant.

Sample[1]	High temperature % germination[2]	Low temperature % germination[2]	Room temperature (control % germination[2]
A[3]	96.3	66.7**	93.3
B[4]	97.0	93.0	-

[1] Variety 569H3 collected on 8-12-1968.
[2] Average of two samples, 50 seeds per sample.
[3] Over 70% of the seedballs straw colored.
[4] Less than 40% straw colored.
**Significantly lower at 1% level.

IV. DISCUSSION

Inhibitory Phenolic Compounds in Sugarbeet Fruits

Results obtained from the study of paper chromatography in connection with germination assay of seedball extracts demonstrated that inhibitors are present in a significant amount to interfere with germination and that the concentration of the inhibiting substances varies from one seed lot to another. These results support the previous work (12, 26, 37). It is also reasonable to assume that the amount of inhibitors may well vary within one variety depending on soil and climatic conditions and the maturity of the seed at harvest.

Earlier interpretations of the control of seed dormancy in sugarbeet assigned particular significance to the physical restriction of pericarp (33). This was justified chiefly by the fact that germination could be induced by rupturing the seedcoats or by holding intact, imbibed seeds under oxygen enriched conditions. The present results (Table 1) show that simple physical restrictions without considering inhibitor action can hardly be justified. From the data presented in this paper it is clear that inhibitors are present in both the pericarp and the caryopsis, and that the type of compounds found are quite similar. The only difference observed is in the concentration of the substances.

Among nine spots to be detected by chromatogram only five of

the spots coincide with those of the cochromatographed reference compounds. Five of the spots are known to be ferulic, p-hydroxybenzoic, vanillic, t-cinnamic and gallic acids. The results confirm the previous reports of other researchers (26, 47). Snyder (37) reported the presence of gallic acid in the sugarbeet fruit. In the present research the concentration of gallic acid was found to be so low that identification of the compound from extracts of small quantities of seed was extremely difficult. Gallic acid was detected only from the extract of ethanol or methanol, and detection from aqueous extract was almost negligible. Furthermore, the experimental evidence (Table 10) indicates that the presence of gallic acid in the sugarbeet fruit can hardly be viewed as an inhibitor of growth or germination in sugarbeet seeds.

From the data shown in Table 8, it is clear that the low germinating variety, 4426, possesses considerably higher concentration of ferulic acid, as well as the other phenolic compounds, compared with normal varieties tested. The results indicate that the concentration rather than type of compound is important in dormancy control in sugarbeet seeds. It is also noteworthy to compare the concentration difference of the seed as well as the pericarp of one variety with that of the other. The concentration difference is much greater in the seeds (caryopsis) of two different varieties than that in the pericarps, which suggests that inhibitors present in seeds may

play a more significant role in dormancy of the sugarbeet seed.

Oxalic Acids as a Germination Inhibitor

The occurrence of oxalic acid and its salts in sugarbeet fruit has been of interest and concern for a considerable time because of its possible role as a germination inhibitor (29, 39). Data shown in Tables 12 and 13 confirms the presence of this organic acid in the seedball. Like phenolic compounds its presence is common in all the varieties tested but the concentration varies from one variety to another. The low germinating variety of 4426 has the lowest oxalate content, which may indicate that oxalates are not involved in seed dormancy. The previous reports (39) that oxalic acid has been related to speed of germination are not substantiated in these tests. It would seem more likely that oxalic acid acts as growth inhibitors as shown by a previous report (39) and by the results presented here.

Germination Inhibitors Defined

The presence of toxic substances in sugarbeet fruit extracts is not necessarily indicative of their involvement in any growth or developmental process. Leopold (22) points out that the presence of cyanide in almonds does not indicate that physiological systems are being inhibited. Any assumption that chemical substances in extracts may be involved in the control of developmental process, therefore,

must be based on evidence that such substances are at least quantitatively related to the process. The chemical substance should also effectively inhibit the particular developmental process, in question. Some of the difficulties may have arisen from the fact that in many instances inhibitors were assayed and studied by a growth test, such as the Avena coleoptile test, a root-growth test, or a test involving the germination of non-dormant seeds which are hardly related to the species. It is clear that many plant tissues, fresh or dry are likely to contain a wide variety of substances capable of inhibiting the growth of Avena coleoptile sections. This method of assay is not sensitive enough for the detection of specific inhibitors which may play a regulatory role in dormancy. The most suitable test, therefore, should be one in which the same species is used both as the source of inhibitor and as the test material. Furthermore, if it is possible to show that the level of inhibitor being studied is correlated with the state of dormancy, it then, may afford a means of distinguishing between substances which are dormancy regulators, and those which are growth inhibitors.

By using sugarbeet seed assumptions to be made in regard to the test materials based on the experimental observations (Tables 18, 19, 22, 23) was that the treated seed has no inhibitory effect during germination.

Although the present data indicate ferulic acid is a major

factor associated with delaying germination, it is not likely that this acid alone exerts a significant inhibition. It, therefore, would appear that either the inhibitory action within the seeds or the fruits is due to the cumulative effects of a number of relatively weak inhibitory compounds. Most of these substances including oxalates as shown in Figure 7 and Table 24 and 25 can be leached out within 12 to 20 hours of running water treatment.

To interpret the functional importance of inhibitors in the sugar-beet fruits, the possibility should be considered that they may be largely present in the tissues not as free acids but as salts, esters, glycosides, and other compounds in a bound form. Mayer and Evenari (28) demonstrated that the inhibiting effect of the derivatives of organic acids is, in general, lower than that of the corresponding free acids. These inhibitors may exert their effects in common with each other and it could be that they are synergistic in promoting inhibition. However, their common effects have not been studied.

Possible Interplay of the Inhibitor and the Promotor

Certain standard procedures including leaching inhibitors and germination at alternating temperatures failed to improve germination, and in some cases actually inhibited germination. These findings support the previous reports by Hoover and Goodin (19). Leaching of inhibitory substances alone does not improve the

germination potential of a given sample lot. The leached seeds must be completely dried for at least several days to give increased germination. Seeds of 20 months drying gave the best germination over the samples of shorter drying period as shown in Table 21 and Figure 6.

Although the presence of inhibitors and their roles are significant, inhibitory substances cannot be separable from other factors involving physiological actions such as growth promotors, and the external environment.

The above phenomenon may well be explained by the regulatory system of an inhibitor-promotor mechanism. Recently, Hashimoto and Rappaport (18) indicated that endogenous gibberellins decrease in naturally maturing bean seeds, but at the same time, neutral substances increase markedly. In a later report, they added that neutral gibberellin-like substances remain unchanged during the bean-seed maturation but increase greatly in mature seeds with a coincident decrease in acidic gibberellin-like components. They conclude that this neutral fraction may constitute a reserve form of gibberellin in dry seeds. Along with these reports, it has been suggested that a great variety of lipids occurring in seeds of a wide range of species may promote inhibitor precursors (1).

From such reports it is becoming increasingly apparent that an active antagonism exists between some growth promotors and

inhibitors in many seeds, although in many instances the specific substances have not been identified.

There is a possibility in treated sugarbeet seed that the promotor-inhibitor complex is shifted to the side which favors promotor level due to excessive loss of inhibiting substances or there may be promoting substances formed from precursors during the drying process.

Comparative Studies on the Inhibiting Compounds and Their Concentrations in Sugarbeet Fruit

A comparison of the inhibitors in variety AH-1 (normal commercial variety) with those from variety 4426 (problem seed of low germination) showed that the inhibitors present in both varieties were almost identical (Table 5). These results indicate that phenolic substances occurring in the sugarbeet fruits are essentially identical from one variety to another regardless of their growing conditions. As noted in Table 8 the concentration of phenolic substances is generally higher in the extract of variety 4426 than in an ordinary sample, which indicates that concentrations rather than the types of substances should be examined.

The importance of concentrations has been stressed by Leopold and Thimann (22, 44). They have stated that many natural inhibitors in plant extracts have a synergistic promotive effect on auxin induced

growth at low concentrations and inhibition at higher concentrations. Such synergistic effects are common to many phenols and flavonoids as well as coumarin (44). The present data suggests that the same principle could be applicable to the germination process in sugarbeet seeds.

Influence of Empties and Hard Seed on Germination

Throughout the germination trials, undeveloped seeds including empties were found quite frequently and usually accounted for three to six percent of most tested samples. The sample of low germinating variety, 4426 has only four to five percent empty seeds. Yet, the results of germination tested by various methods showed a germination total of only 50 percent for this seed (Table 22). By leaching and drying technique the germination potential of this sample can be increased to nearly 80 percent. At the end of the germination, the remaining ungerminated seeds were examined one by one by the cutting method (43). The ungerminated seeds from treated samples are either empties, decayed, or partially developed seeds (Table 22). This experiment again shows that the leaching and drying process completely eliminates the segment of ungerminated seeds in any given sample which could be classified as hard seed.

Functions of Germination Inhibitors in Sugarbeet Seed

The usual function of germination inhibitors is to delay germination or to induce dormancy. The question arises as to the role of inhibitors in the dormancy of sugarbeet fruit where the testa interferes with gaseous exchange or where there is a requirement for certain specific environmental conditions, such as a period of leaching or chilling. This problem will now be briefly discussed.

Dormancy of the sugarbeet seed is due to the relative impermeability of the seedcoat to oxygen. This conclusion is based mainly on the following observations.

1. Sugarbeet seed will germinate readily under submerged conditions (Table 1) when supplied with enough oxygen or air.
2. Seed removed from the pericarp germinates rapidly.
3. Unleached seeds require very high amounts of oxygen as compared to leached seeds (Figure 10).

Seeds contain water soluble inhibitors and these are readily leached out in the absence of the seedcoat but the presence of the seedcoat interferes with the ability of water to reach the sites of inhibiting substances. As a result of this impermeability to water the loss of inhibitors from the seed itself requires a considerable leaching period. For this reason, among the inhibitors of the sugarbeet fruit, those present within the seeds may be far more important

in dormancy control than the substances in the seedcoat which can be readily washed out.

The original seeds of variety 4426, removed from the testa, showed considerable delay in germination compared with treated seeds of the same variety, which may indicate the disappearance of some of the inhibitors from the treated seeds by leaching.

Experimental evidence indicates that dormant sugarbeet seed can be induced to germinate, either by leaching out the inhibitors, or by placing them in an atmosphere of high oxygen tension. The oxygen requirement of the original seeds is also higher than that of leached seeds (Figure 7). The conclusion can be reached that oxygen is directly related not only to the germination of seed but also for the oxidation of inhibiting substances. Similar experimental evidence was reported by Wareing in his work with Xanthium seed (52). He found that when seeds were placed in an atmosphere of 100 percent oxygen, there was a rapid decrease in the inhibitor content, even before there were any visible signs of germination. Similar affects have been observed in Avena fatua by Black (6).

The increased germination of sugarbeet seed when treated with hydrogen peroxide (43) may be explained by the same basic mechanism observed by the above researchers. Hydrogen peroxide is an oxygen source and can create high oxygen concentrations which in turn aid the germination process by breaking down inhibiting

substances and also by supplying necessary oxygen to the germinating seed.

The Role of Other Factors Influencing Low Germination

Several researchers including MacKay et al. (24) have reported the influence of seed borne pathogens on sugarbeet seed germination. It is not likely that seed-borne pathogens are directly involved in the germination process. If pathogens exert an effect, it may occur after germination or in the seedling stage. During the course of the present research it has been observed that many good seed lots always germinate well even under heavy fungus infestation, although the seedlings were destroyed by the pathogenic attacks in the later stages. The effects of pathogens on the sugarbeet germination may well be a minor problem as compared to other factors.

The process of decorticating sugarbeet fruit is known to be beneficial. This technique is often used by companies for two reasons: The process helps is sizing seed for precision planting and increases germination by removing some of the inhibitors and pathogens present in sugarbeet fruits. However, when the mechanical damage caused by decortication is considered, this process may not in itself lead to better germination and is probably not a valid solution to stand improvement.

Leaching and Drying Effects on Seed Germination and Seedling Vigor

Germination potential of variety 4426 can be increased by more than 20 percent (from 56.0 to 79.0%) by the process of drying after leaching as shown in Figure 6. Speed of germination and seedling vigor measured by the growth rate, also showed large increases. The inhibiting substances in the seedcoat may be washed out within a short period of leaching process, but the phenolic inhibitors within the seed may not be readily removed. As a result, fruits containing large quantities of phenolic substances within the seed require a considerably longer period of leaching than the normal seed lot.

From the results shown in Figure 7, it can be observed that many substances, including soluble oxalate disappear, as the leaching period is extended. Among these substances only oxalic acid and ferulic acid were made a quantitative correlation with the period of leaching. Further study is needed for valid quantitative determinations of other compounds removed by leaching.

In comparing overall results of the germination trials, the leaching-drying process appears to offer the maximum benefit for improving a given sugarbeet sample. No exceptions have been observed throughout the germination experiment of all the varieties tested, including variety 4426. All the germinable seeds including hard seeds can be germinated by this method.

Increased speed of germination, and seedling vigor have been observed in all samples treated by leaching and drying. Eighty percent or more of the germinable seeds of a treated sample will germinate within two to three day periods while the original sample requires four to five days to reach 80 percent mark.

The data on leaching and drying shown in Tables 19, 21 and 23 indicate that in the varieties tested (AH-1, HH-5 and USH-9B) a 12 to 16 hour-leaching period is most desirable considering such factors as speed of germination, total percentage germinated, and seedling vigor. Quite possibly some seed lots may require a longer time compared with other seed lots depending upon the inhibitor content within the fruits. The optimum leaching time for a sample of 4426 was 48 hours as shown in Table 22 and Figure 6. With the ordinary commercial varieties a 12 to 20 hour-leaching period is satisfactory. Thirty hours or more leaching will not change germination but at the later stage of seedling growth loss of vigor has been observed. This may indicate a loss of promoting substances along with inhibitors due to excessive leaching, although this loss of vigor is not great.

Evenari (14) reported that prolonged leaching (by excessive rain) strongly decreased germination. According to his explanation, the decreased germination was due to several factors: 1) Over-leaching reduces the concentrations of inhibitors below that required for stimulation (low concentration of the inhibitor stimulates

germination). 2) Both promotors and inhibitors in the fruits are leached out at different rates and over-leaching removes both the inhibitor and the stimulator. The present research has not evaluated leaching to the point of over-leaching, but the results shown in Table 15 may support his findings.

The problem of undeveloped seeds (empty) and abnormal seed has not been studied. Hills reported that lygus bugs caused empty seedballs by feeding on the developing seed during seed maturation. Other suggestions on the causes include parthenocarpy, pollination failure, nutritional deficiencies, and environmental effects; however, no conclusive evidence has been presented as to the causes of undeveloped seeds.

According to Tekrony (43), the number of undeveloped seeds found in Oregon grown commercial varieties ranges from 4.5 to 17.5 percent with an overall mean of 7.5 percent. Based on his results, these varieties should give results of germination from 82.5 to 95.5 percent with an overall mean of 92.5 percent if undeveloped seeds (empty seeds) were the major cause of germination failure. Actual laboratory germination results, according to his data, showed considerably lower germination percentage than can be accounted for by empty seeds. These were 82.58 percent with H_2O_2 method, 74.87 percent with standard method and 83.67 percent with field emergence.

There is an overall average of more than ten percent difference

between the expected 92.5 percent and the average of the actual results obtained by these methods.

During this investigation no seed lot has been found that had more than six percent undeveloped seeds. Most varieties ranged from three to five percent undeveloped seeds. This 10 to 20 percent margin, then, may well be classified as hard seeds and (or) seeds with inhibitors. As shown in Tables 18, 19 and 23 a major portion of germination failure could be eliminated by leaching and then drying a given seed lot. If the seed industry can devise a way of using this technique, it could have practical importance. Coating the sugarbeet fruits with an absorbant such as clay or activated charcoal after the leaching and drying treatment, may facilitate sizing of the seedball for precision planting.

V. SUMMARY AND CONCLUSIONS

An investigation was conducted into the causes of low germination of Oregon grown monogerm sugarbeet seed with emphasis on chemical inhibitors present in fruits.

The results shown by chemical analyses indicate that ferulic acid is present in all the varieties tested. This acid acts as a strong inhibition on germination and growth of seedlings in a greater range of concentrations than other phenolic compounds.

Pericarps as well as seed of sugarbeet contained nearly identical phenolic compounds and it appears that the concentration rather than the kinds of chemicals present has a significant influence on dormancy. Among the varieties tested, the poor germinating variety, 4426 has a higher concentration of phenolic substances, particularly within the seeds. This may indicate that the inhibitor concentrations within the seed have a greater influence on dormancy control than those in the pericarp.

The presence of oxalates in the seedball apparently did not effect germination but exerted a toxic effect on the newly emerging primary root.

Maturity studies show that early harvested samples give higher and faster germination than those from late harvests which might indicate that the inhibitors are formed within other parts of the plant

organs and moved to the fruit as the seed matures.

Various methods were tested to improve germination of monogerm varieties. Complete drying of seed after leaching gives the best germination results of all methods tested. This leaching and drying process has been shown to be the most satisfactory way to get the maximum germination potential from a given lot or sample.

This technique may be applicable to commercial practice after the technical and economic aspects of the procedure are worked out.

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APPENDIX

Appendix Table 1. Analysis of variance for the effect of germination medium on sugarbeet seed germination.

Source of variation	Original seed			Treated seed		
	df	Sum of square	Mean square	df	Sum of square	Mean square
Among treat	6	106	17.67	6	8	1.33
Error	21	212	10.10	21	67	3.19
Total	27	318		27	75	

Appendix Table 2. Analysis of variance for the influence of several solvent extracts of sugarbeet fruit upon germination of treated monogerm variety 569H3.

Source of variation	df	Sum of square	Mean square
Treatment	7	261	37.29**
Error	16	281	17.80
Total	23	542	

**Significant at 10 percent probability level.

Appendix Table 3. Analysis of variance for the effects of flavonoids from sugarbeet fruit on seed germination.

Source of variation	df	Sum of square	Mean square
Treatment	5	947.8	189.56**
Error	12	194.6	16.22
Total	17	1,142.4	

**Significant at one percent probability level.

Appendix Table 4. Analysis of variance for the effects of aqueous extracts of pericarp and seed on the germination of sugarbeet seed (variety 569H3).

Source of variation	df	Sum of square	Mean square
Treatment	3	214.53	214.53**
Error	8	3.68	3.68
Total	11	218.21	

**Significant at the one percent probability level.

Appendix Table 5. Analysis of variance for the effects on germination and seedling vigor of pure chemical solutions known to be present in sugarbeet seed as germination inhibitors.

Source of variation	df	Sum of square	Mean square
Treatment	8	62.31	7.79**
Error	27	106.00	3.92
Total	35	168.31	

**Significant at the ten percent probability level.

Appendix Table 6. Analysis of variance for the comparison of leaching vs. soaking effects on the germination of monogerm sugarbeet seeds.

Source of variation	Sample A			Sample B		
	df	Sum of square	Mean square	df	Sum of square	Mean square
Treatment	2	971.67	485.84**	2	1,078.64	539.32**
Error	6	116.83	19.47	6	159.32	26.55
Total	8	1,088.50		8	1,237.96	

**Significant at the one percent probability level.

Appendix Table 7. Analysis of variance for the effects of leaching treatment upon the germination of monogerm sugarbeet varieties.

Source of variation	Original seed			Treated seed		
	df	Sum of square	Mean square	df	Sum of square	Mean square
Among variety	3	118.74	39.58	3	78.63	26.21
Error	8	320.00	40.00	8	53.44	6.68
Total	11	438.74		11	132.07	

Appendix Table 8. Analysis of variance for the comparison of treated and untreated samples of variety 569H3 harvested at different times.

Source of variation	Original seed			Treated seed		
	df	Sum of square	Mean square	df	Sum of square	Mean square
Harvest dates	3	350.0	11.67	3	67.7	22.57
Error	16	100.0	6.25	16	134.3	8.37
Total	19	450.0		19	202.0	

Appendix Table 9. Analysis of variance of the effects of leaching and drying on the germination of sugarbeet seeds variety 569H3 harvested from a single plant.

Source of variation	df	Sum of square	Mean square
Treatment	2	1,126.0	563.08*
Error	6	75.0	12.5
Total	8	1,201.0	

**Significant at the one percent probability level.

Appendix Table 10. Analysis of variance for the comparison of germination after several pre-germination treatments for sugarbeet variety of 4426.

Source of variation	df	Sum of square	Mean square
Treatment	4	3,705.6	926.4**
Error	10	328.0	32.8
Total	14	4,033.6	

**Significant at the one percent probability level.

Appendix Table 11. Analysis of variance for the effect of leaching time on the germination of sugarbeet seed (variety AH-1).

Source of variation	df	Sum of square	Mean square
Treatment	5	37.12	7.42
Error	12	58.56	4.88
Total	17	95.68	

Appendix Table 12. Analysis of variance of the effects of gibberellic acid on the germination of monogerm sugarbeet seeds.

		Original seed			Treated seed		
	Source of variation	df	Sum of square	Mean square	df	Sum of square	Mean square
USH-9B	Treatment	2	33	16.5	2	14.3	7.15
	Error	6	133	22.2	6	298.7	49.78
	Total	8	166		8	313.0	
4426	Treatment	2	92	46.0**	2	11.5	5.75
	Error	6	24	4.0	6	10.7	1.78
	Total	8	116		8	22.2	

**Significant at the 5 percent probability level.

Appendix Table 13. Analysis of variance for the influence of drying temperature following harvest upon the germination of sugarbeet seeds collected from a single plant.

Source of variation	df	Sum of square	Mean square
Treatment	2	1,600.2	800.1**
Error	6	22.0	3.67
Total	8	1,622.2	

**Significant at the one percent level.